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THE W. MORTON GRANT

Proctor Medal Award

PROCEEDINGS

of the

Association for Research in Ophthalmology, Inc.

Twenty-fifth Meeting

Chicago, Illinois

June 11, 12, 13, and 14, 1956

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RESEARCH IN OPHTHALMOLOGY, INC.

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W. Morton Grant, M.D.

REMARKS ON ACCEPTANCE OF THE PROCTOR MEDAL AWARD

June, 1956

W. Morton Grant, M.D.
Boston, Massachusetts

To this association and to the committee charged with carrying out the wishes of Dr. Proctor, I give my thanks for this award.

I understand that on this occasion I am also given license to put on record a few words, and, since I certainly shall never again have such a wonderful chance, I wish to take this opportunity to pay tribute to three men for whom I have a particular admiration and to whom I have reason to feel most grateful.

These men are known so well to so many that it might seem superfluous to speak of them before ophthalmologists who are their contemporaries. However, for ophthalmologists of the future and for those of the present who may know these men more for their scientific contributions than for their personal attributes, I would like to get on the record a few words about their personalities and characters as we have the privilege to know them. Perhaps I have some advantage in undertaking this characterization because of my good fortune in having had personal associations with these three men over a period of 15 years. I wish I also had some advantage in ability to describe them.

In order of seniority and foremost in candor stands Frederick H. Verhoeff. To me he is most admirable for his out-spoken honest expression of opinion. He does not defer to rank or personal feelings; although in the case of a novice he does usually soften his blows. He has an extraordinarily analytical and inventive mind, which even in his eighties appears in no way slowed. Characteristically, when discussing a paper which he has just heard for the first time he can be counted on to have formulated an opinion, to have ready pertinent searching questions, and to have considered aspects and possibilities which the author of the paper usually has not considered. It is seldom that he has occasion to admit that he has no comment or that this is not a subject on which he has already written a paper in 1906. He zealously guards credit for originality, whether for himself or for others. His criticisms and comments are expressed with subtle humor rather than rancor, unless he feels some injustice has been done. In any case a candid expression of opinion can be counted on. He is more ready to accept with enthusiasm new ideas that are backed up by good evidence than are most men many years his junior.

Dr. Verhoeff's visits to the laboratory in which I work have usually held for me some threat of embarrassment, as well as considerable promise of stimulation. He regularly embarrasses me by being better informed than I am on the very latest develop-

ments in the field of my own research, and then makes me feel worse by questioning me on points in my work which I have neglected to settle. This generally is a stimulus to try to do better, after recovery from the initial blow, of course.

The center man of the triumvirate for whom I am proud to express my special admiration is Dr. Paul A. Chandler. He most certainly has endeared himself to many of you not only through his sincerity and warmth of personality but through his tireless teaching and valued clinical counsel. Although he is constantly under great pressure from the many demands upon him, I have never known him to lose his great sense of humor or to become short-tempered even under the most trying circumstances. Even when an inexperienced surgical nurse roughly dropped his new cataract knife into a pan along with other instruments the extent of his reaction, as I recall, was to remark a little sadly that he guessed that was the end of that knife. He has the greatest consideration for the feelings of others, and never lets himself seem too rushed to give his best attention and thought to the many so-called curbstone consultations which are thrust upon him.

To me, one of Dr. Chandler's most pleasing and remarkable attributes is his deep and inquiring interest in mechanisms underlying clinical phenomena, particularly in the mechanisms of intraocular pressure and glaucoma. I have occasion to be repeatedly impressed with his extraordinary faculty for sharing with the laboratory-investigator his interests and enthusiasms, and with his faculty for translating the products of basic research into clinical significance and application. I believe it is rare for a man who is primarily a clinician to have such true insight into research as does Dr. Chandler.

Third in my triumvirate of most admirable men, but third only because he is junior to the others, is Dr. David G. Cogan. Of him I must speak more guardedly, since he in particular is in a position to make me wish to weigh my words quite carefully. With due reflection, then, I think of him as a thoroughly honorable man gifted with a great sense of humor and a zest for work and for enjoying life. Like Dr. Chandler, he is considerate and sympathetic to the shortcomings of others. To this I can certainly attest from personal experience.

From a scientific aspect I have long been impressed with Dr. Cogan's faculty for deriving fundamental concepts from simple experiments. In this day in which so many investigators have come to rely upon expensive and elaborate equipment to furnish them information on details, Dr. Cogan succeeds with a minimum of artifice to recognize principles. Yet, as an exemplary director of research, he appreciates the needs of the rest of us for assistance from the fancy equipment.

As a scholar, too, he is somewhat amazing. Though possibly best known in the roles of ophthalmic physiologist, pathologist, and neuro-ophthalmologist, I have discovered that he regularly wakes at five in the morning and studies biochemistry.

In closing my little tribute to these three men, I wish to assure you that among their associates are many other admirable men, but these three I personally put at the top of my list.

BIOGRAPHICAL DATA

W. Morton Grant, M.D.

Morton Grant was born July 12, 1915, at Lawrence, Massachusetts. Here and in the adjacent town of Andover he spent his boyhood, attending the public schools through the seventh grade, New Hampton School at New Hampton, New Hampshire, for his eighth grade, and Philips Academy in Andover for his high school years. He was graduated from Harvard College in 1936 and from Harvard Medical School in 1940.

Dr. Grant spent one year as a surgical intern at Henry Ford Hospital in Detroit and then, with the onset of the Second World War, was drafted into the war-time researches of the Howe Laboratory of Ophthamology. He has been a staff member of this laboratory ever since, now holding the appointment of associate professor of ophthalmology of the Harvard Medical School and assistant surgeon at the Massachusetts Eye and Ear Infirmary.

Married to Jeanette Poirier in 1936, Morton Grant has had a loyal and sympathetic helpmate throughout his medical school and subsequent years. The immediate family now holds to its great credit three children: David, aged 13 years, and the twins, Jean and Jeff, aged 11 years.

Born into a physician's family, Dr. Grant logically went into medicine but his native interests have always inclined him to chemistry. This was early evident when, as a boy, he is said to have developed some pyrotechniques that caused great concern to the neighborhood. In the years of his preparatory school he was top man in his chemistry class and in college he was permitted to do advanced work in organic chemistry while still an undergraduate. Whether to pursue a career in chemistry or to go into medicine was no little problem. The precedent and advice of his father decided him in favor of the latter, and Dr. Grant's subsequent life has proved that the two fields are not mutually exclusive.

Dr. Grant's talents have always flourished in an atmosphere of freedom. Probably the most significant period in his entire training was a year that his parents, with admirable understanding, permitted him to leave school and set up a course of self-instruction. The effectiveness of this year is attested by the fact that most of his early experiments in chemistry were done in a home-made laboratory during this time. His entire precollegiate training in physics was acquired at home and yet his subsequent standing in the entrance examinations was such as to enable him to enter college with only three years of preparatory school.

Dr. Grant's interest in ophthalmology was initiated in considerable measure by Dr. Z. W. Colson who, in turn, had specialized in ophthalmology at the suggestion of the senior Dr. Grant. While in medical school, Dr. Morton Grant elected additional courses in ophthalmology and came in contact for the first time with ophthalmic research. After finishing his surgical internship, he returned to ophthalmic research in earnest. By force of circumstances he was first concerned with the effects of mustard gas on the eyes and on tissue in general. Together with Dr. Kinsey, he was the first to develop a test for the persistence of mustard gas in tissue. Subsequent studies have centered chiefly but not exclusively about the fields of aqueous humor dynamics, pharmacology of the eye, and toxicology of the eye. Most recently he has developed the method and theory of tonography which has had world-wide ramifications. Withall he has been actively engaged in clinical and surgical activities at the Massachusetts Eye and Ear Infirmary and teaching in the postgraduate courses at the Harvard Medical School.

Not evident in the foregoing vital and academic statistics but nonetheless essential to even this brief characterization are the following:

Dr. Grant is modest to an unusual degree and sharply critical of his own accomplishments. He shuns publicity and has little respect for those who make publicity an end in itself. He has set high standards, ethically and intellectually, for himself and for others. While generally taciturn, he speaks well and at length when the occasion calls for it. But most noteworthy, perhaps, is Dr. Grant's independence of thought and action. This characteristic which may well underlie his original, and at times unorthodox, approach to problems is perhaps best exemplified by the fact that he has had no formal ophthalmic training although he is an astute and much consulted clinician. Dr. Grant's accomplishments are refreshing evidence that native initiative and curiosity can still supersede organized training.

243 Charles Street (14).

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REACTIONS OF THE ELEMENTS OF RETINA AND OPTIC NERVE*

In common morbid entities of the human eye

J. Reimer Wolter, M.D.
Ann Arbor, Michigan

Introduction

Pathologic alterations of the human retina and optic nerve in common morbid entities of the eye are composed of certain specific and/or nonspecific changes of cells and cell elements. A study of these fundamental cellular reactions is important since it helps to understand the development and nature of the more complex pathologic situations.

Typical pathologic reactions of the astroglia and other glial elements of the human retina were presented last year at this congress.[1] The present demonstration is a continuation of that study; it is concerned with the degenerative changes of neuronal elements, the pigmented epithelium, and the blood vessels of the human retina. A description of some types of degeneration of the nerve fibers of the optic nerve, in pathologic conditions, is included.

Material and Method

The human eyes used in this study were obtained either after surgical enucleation or at post mortem. All eyes were fixed in formalin or bromformalin and were divided horizontally through the posterior pole. One half of each eye was imbedded in paraffin, cut in serial sections, and stained with hematoxylin-eosin. Flat sections of the isolated retina and choroid and longitudinal sections of the optic nerve were made on the freezing microtome from the other half of each eye. The silver carbonate methods of del Rio Hortega[2] were used to stain these sections. These techniques have proved to be very useful in demonstrating histologic details of the tissues of the human eye under normal and pathologic conditions.[1, 3-7] All illustrations contained herein are unretouched photomicrographs.

Histologic description

The multitude of the histologic details and the limitation of space restrict this study to some typical examples.

1. Typical pathologic changes of the neurons of the innermost three layers of the retina

Cytologic study of the neurons of the inner layers of the human retina, in common morbid entities, revealed only degenerative

* From the Laboratory of Neuropathology and Neuro-ophthalmology, Neuropsychiatric Institute, University of Michigan Hospital. Supported by Grant No. B475—C 2 of the United States Department of Health, Education, and Welfare.

changes. No hypertrophy, proliferation, or true regeneration of damaged or destroyed ganglion cells were observed.

The pattern of the above-mentioned degeneration of these retinal ganglion cells and their processes mainly depends on the severity and the duration of a causative agent. But it is similar in many different pathologic situations.

Invariably, severe acute damages of different origins immediately destroy the inner neurons in the same manner. There is dissolution of all ganglion cells and their processes without visible histologic signs of active reaction. Such acute damage is known to occur in large retinal areas; for example, in embolism of the central artery or in some cases of acute panophthalmitis. This is characterized by the breakdown of ganglion cells. Their remnants are phagocytized by microglia which become swollen and loaded with lipoid substances. These are then called "gittercells" or "granular compound corpuscles." They carry the debris of the destroyed neurons to the blood vessels. Hypertrophic astroglia finally fill the space of the phagocytized neurons with dense glial scar tissue. If, however, the acute damage results in a destruction not only of the ganglion cells but also of the astroglia, cystoid degeneration (status spongosus) of the retina develops. In such a state there are large, cystlike spaces and the retina then is represented only by a loose framework of surviving elements of Müller and connective tissue. If the damage is more severe the entire retinal structure may disintegrate.

In more chronic situations associated with slight alterations, the retina finds time for slowly developing reactive and degenerative changes of the ganglion cells. All stages of these changes can be studied in histologic sections of eyes. Such reactive and degenerative changes may be observed as morphologic alterations of shape and structure of the cells and their processes. They usually represent a slowly progressive destruction of the neurons. Remissions, however, often occur in such diseases. Improvement of the metabolic situation of the neurons may result and permit temporary survival of the damaged cells and even attempts at cellular regeneration.

The first pictures represent typical examples of degenerative changes of neurons in the ganglion cell layer in chronic disorders of human eyes (figs. 1-8).

Figure 1 shows a neuron of the ganglion cell layer of a blind eye with secondary glaucoma following old perforating corneal injury. The cell body with its dendrites (b) is swollen and clearly shows beginning dissolution. The coarser neurit (axon) (a) is coiled in a bizarre pattern. Normally such cells have a sharply outlined cell body, very delicate dendrites, a straight regular neurit, and a distinct nucleus. The bizarre coiling and malformation of the neurit, the swelling and interruption of the dendrites, and the beginning of dissolution of the cell body and nucleus are some of the typical signs of permanent degeneration of retinal neurons.

Figure 2 shows a neuron of the ganglion cell layer of the retina of a blind eye with an old retinal detachment. The cell body (b) and the dendrites (c) show malformation and are swollen. The cell limits are sharp and the nucleus is visible (a). This cell has suffered severe damage; for without the neurit, it is worthless for visual function. There are no signs of regeneration; the cell therefore merely exists.

Figure 3 shows two neurons of the ganglion cell layer of a case with absolute, primary glaucoma. The neurites, here, have also disappeared but the cell bodies (a-b) and some dendrites (c) have survived. These cells show very advanced atrophy, the left one particularly is only a shrunken remnant of a neuron and should be considered only as detritus. In chronic diseases, where the direct damage is not severe enough to destroy the neurons at once, such slowly developing atrophy of the cell bodies often occurs following breakdown of their processes. These cell bodies may survive for a long

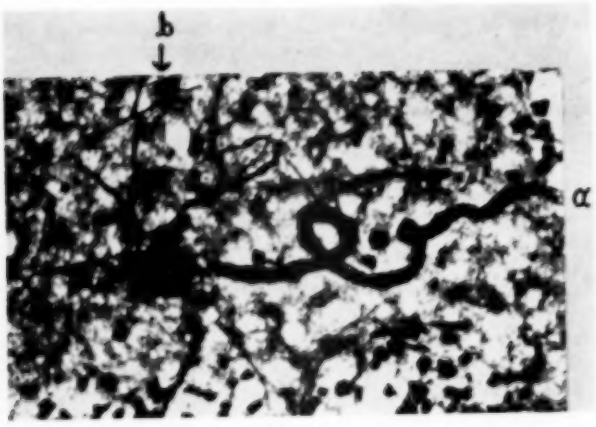

Fig. 1 (Wolter). Neuron of the ganglion-cell layer of a blind eye with secondary glaucoma following old perforating injury. The cell body and its dendrites (b) show swelling and dissolution. The neurit (a) is coiled up. (Hortega method, photomicrograph, ×700.)

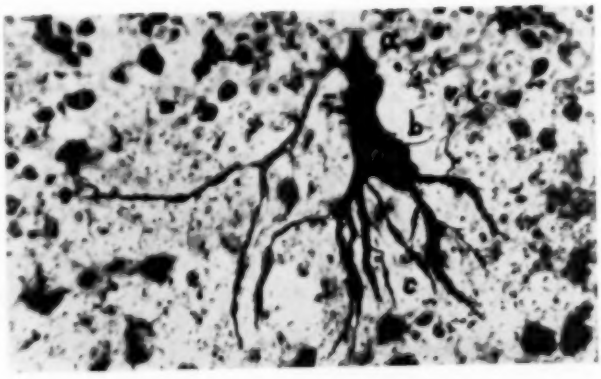

Fig. 2 (Wolter). Neuron of the ganglion-cell layer of a blind eye with old retinal detachment. (a) Stump of neurit, (b) cell body, (c) dendrites. (Hortega method, photomicrograph, ×700.)

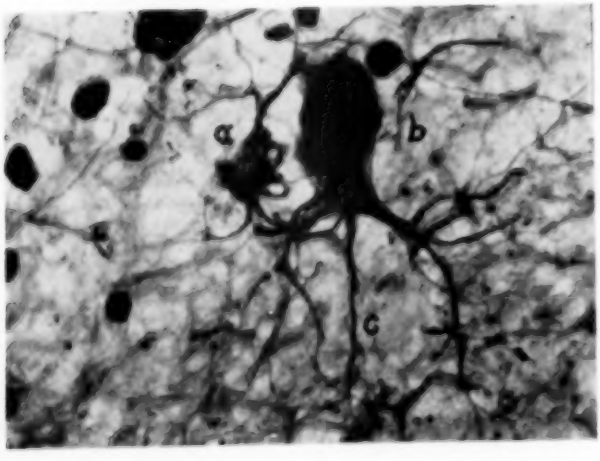

Fig. 3 (Wolter). The remnants of two neurons of the ganglion-cell layer (a and b) of an eye with absolute, primary glaucoma. (Hortega method, photomicrograph, ×700.)

time, but are worthless for any visual function and will finally disappear.

Figure 4 shows another form of atrophy which often occurs in neurons of the ganglion cell layer in chronic disorders of the eye. The neuron in this picture and all its processes have essentially their normal shape. The whole cell is only faintly visible, has very diffuse structures, and no sharp limits. The neurit (a) has almost completely disappeared. In this type of degeneration there often occur extensive tigroid granula (Nissl) in the protoplasm of the ganglion cells. Such atrophy may be seen in many different chronic situations together with other forms of degeneration in the same eye. Figure 4 is taken from a section of the retina of an eye with secondary (hemorrhagic) glaucoma.

Figure 5 represents a very late stage of degenerative atrophy of retinal ganglion cells in an eye demonstrating chronic uveitis following corneal laceration. Only the shrunken remnants are left of two neurons of the ganglion-cell layer.

Figure 6 demonstrates that there really is no definite rule for the development of degeneration of these neurons in chronic eye diseases. Usually, the dissolution of a neuron begins at the processes and the cell body survives for some time after their disappearance. However, this example from the retina of an eye with primary absolute glaucoma

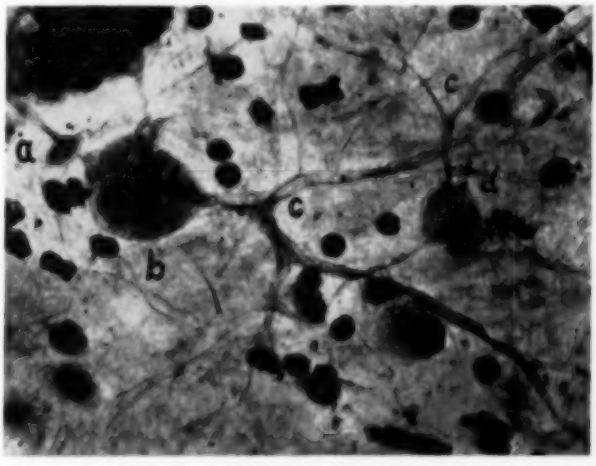

Fig. 4 (Wolter). Two atrophic neurons (b and d) of the ganglion cell layer of an eye with secondary hemorrhagic glaucoma. (a) Neurit, (b) and (d) cell bodies of ganglion cells, (c) dendrites. (Hortega method, photomicrograph, ×700.)

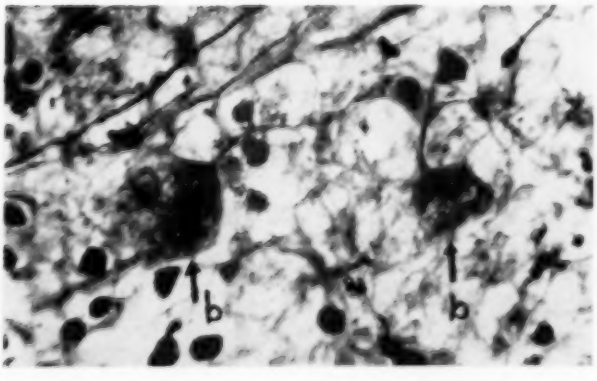

Fig. 5 (Wolter). Late stage of degenerative atrophy of two neurons of the ganglion-cell layer of an eye with chronic uveitis following corneal laceration. (b) Remnants of the cell bodies. (Hortega method, photomicrograph, ×700.)

shows the very well-preserved dendrites of a neuron, the cell body and neurit of which have disappeared. This certainly would not be considered true survival of the dendrites since such processes are not able to live without the cell body. Generally, parts of neurons—for example, fragments of neurites, naked nuclei, or fragments of dendrites—may be seen in the tissue for quite a while after the breakdown of neurons in many advanced pathologic conditions of the retina (necrotic preservation of Cajal).

Neurons react with very similar types of degeneration to many kinds of pathologic involvement. These highly differentiated cells show surprisingly similar types of reactions to a wide variety of common pathologic damages.

In the following, some types of degeneration will be demonstrated which were commonly seen in the ganglion cell fibers of the nerve fiber layer. The latter represent the neurites of the neurons of the ganglion cell layer.

Figure 7 represents a horizontal section through the nerve fiber layer of the retina of an eye with absolute glaucoma. It shows nerve fibers, all of which are swollen and irregular in shape. Two of them are interrupted and their distal stumps show coarse, bulblike terminal swellings (a). The occurrence of these terminal swellings and the interruption of the nerve fibers are part of the slowly descending retrograde degeneration in the optic nerve and in the nerve fiber layer of the retina. The more proximal parts of the neurites which ran to the disc of the optic nerve and on to the brain are destroyed by "Wallerian" degeneration and completely removed.

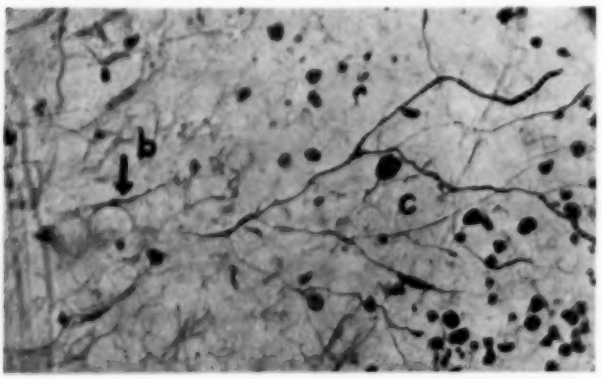

Fig. 6 (Wolter). Well-preserved dendrites (c) of the ganglion-cell layer of an eye with primary absolute glaucoma. The cell body (b) which belonged to this process has disappeared already. (Hortega method, photomicrograph, ×700.)

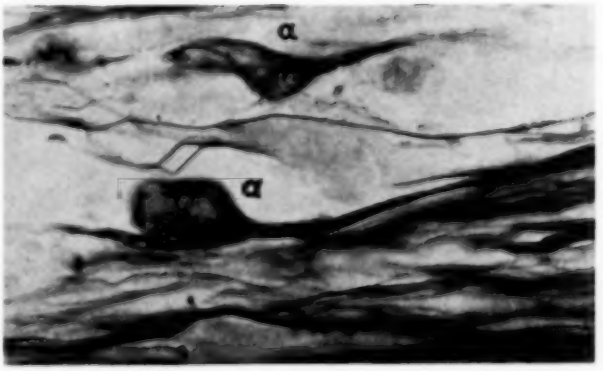

Fig. 7 (Wolter). Horizontal section through the nerve-fiber layer of a retina with absolute glaucoma. Two nerve fibers (a) show retrograde degeneration and typical terminal swellings. (Hortega method, photomicrograph, ×800.)

Such bulblike swollen stumps of partly destroyed neurites were seen in great numbers in the nerve-fiber layer of the retina of many pathologic eyes with chronic involvement of the retina. Most typically, very numerous terminal swellings of this type develop in glaucoma or in a certain stage of degeneration in retinal detachment.

Bulblike terminal swellings of nerve stumps in peripheral nerves in men and animals were first described and extensively studied by Cajal[8] and called by some authors the "degeneration-bulbs of Cajal."

Figure 8 also shows some typical bulblike, terminal swellings of fibers of the nerve fiber layer of the retina in a case of old retinal detachment. Besides these terminal bulbs, in this case, there are also single round bodies of lipoid material which represent the remnants of such terminal swellings after the breakdown of the distal part of the nerve fiber. All stages of the process from degenerating nerves, with terminal swellings, to these single, round lipoid bodies without any connection to nerve fiber structures can be observed.

In eyes with retinal detachment, there may be seen not only retrograde degeneration of nerve fibers but also attempts at regeneration. As an example, there is illustrated in Figure 8 (b) an interrupted nerve fiber, the distal end of which shows the bizarre formation of a coiled-up axon. This finding perhaps indicates that, shortly before the enucleation of this eye, there must have been an improvement of the nutrition of this particular neuron. Consequently there occurred budding of this distal stump of the interrupted nerve fiber and the formation of the bizarre, coiled, new-grown axon. This finding clearly represents an attempt of regeneration of neurites and is to my knowledge the first actual demonstration of any regeneration in retinal neurons of the adult man.

Figure 9 shows a flat section of the nerve fiber layer of the clinically normal eye of a 76-year-old man. Within one of the nerve fibers there is a large, lamellary arranged, round body which clearly is surrounded by a thin wall of structures of the nerve fiber. This round body is a formation of hyaline substance. Such hyaline bodies in nerve fibers were seen very often in the retina of old men and also in pathologic conditions. Such, apparently, represent a typical form of degeneration of retinal nerve fibers. This kind of degeneration may be observed in all stages of its development and the end-result is the complete disappearance of the nerve fibers while the hyaline bodies remain in the nerve fiber layer. In the literature, such hyaline formations have been called "ganglioforme swellings" or "cytoid bodies."

Various other kinds of hyaline degenera-

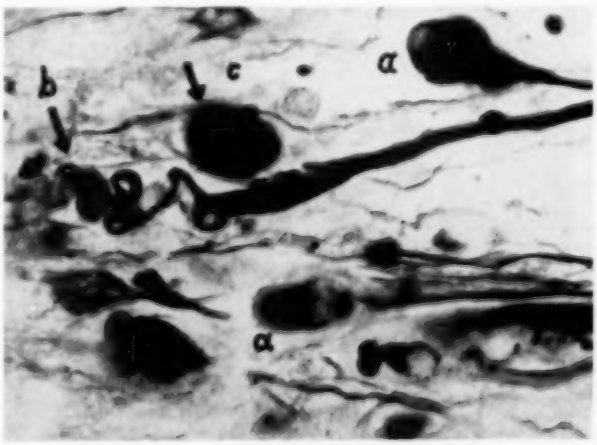

Fig. 8 (Wolter). Horizontal section through the nerve-fiber layer of an eye with old retinal detachment. There are terminal swellings of degenerating nerves (a), an attempt of regeneration of a nerve fiber with the formation of a coiled, new-grown axon (b), and round lipoid bodies without connection to nerve fibers (c). (Hortega method, photomicrograph, ×800.)

tion of different elements of the human retina will now be discussed.

2. Hyaline degeneration of elements of the human retina and choriocapillaris

It was shown above that small hyaline bodies may develop from degenerating neurites in the nerve-fiber layer. Degeneration of astrocytes of the retina, however, may in addition result in the formation of very similar, round hyaline bodies.

Figures 10 and 11 are taken from the retina of a typical, advanced case of retinitis pigmentosa.

Figure 10 shows an astrocyte of the retina (arrow), the processes of which have

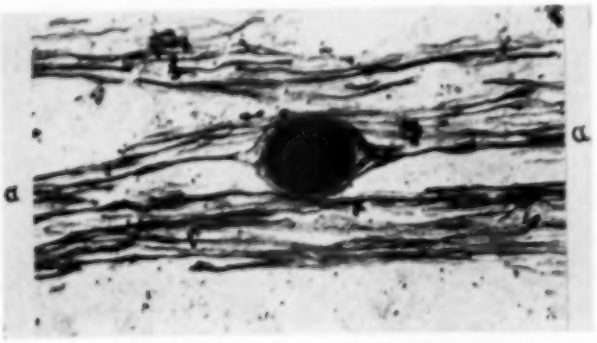

Fig. 9 (Wolter). Horizontal section through the nerve-fiber layer of the eye of a 76-year-old man. One nerve fiber (a-a) contains a large, round hyaline body. (Hortega method, photomicrograph, ×800.)

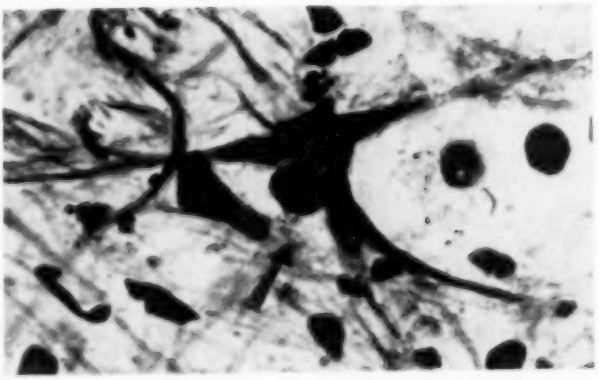

Fig. 10 (Wolter). Star-shaped, partly degenerated body of an astrocyte (arrow) of the ganglion-cell layer of an eye with advanced retinitis pigmentosa. (Hortega method, photomicrograph, ×800.)

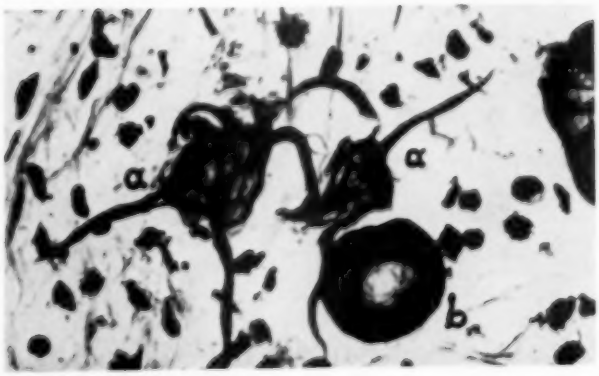

Fig. 11 (Wolter). More advanced stages of degeneration and hyalinization of astrocytes of the ganglion-cell layer of the same retina with retinitis pigmentosa (compare Figure 10). (a) Hyalinized astrocytes which still have some remnants of the processes. (b) Round hyaline body which represents the end-result of hyaline degeneration of astrocytes. (Hortega method, photomicrograph, ×700.)

been destroyed. The star-shaped body with the eccentric nucleus is still clearly visible.

Figure 11 shows even more degenerated astrocytes of the same retina which are in late stages of hyaline degeneration. In this picture, two of the cells have short remnants of their processes, are hyalinized, and the nucleus is no longer visible. A third cell represents the end-result of this type of degeneration, a single, round hyaline body (b) which has about the same size and structure as those of nerve-fiber origin described above. All stages of the development of such hyaline bodies from these astrocytes can be observed. Hyaline degeneration of astrocytes has also been seen in other slowly progressive degenerations of the retina. It can also be occasionally seen in the normal aging eye.

Under rare conditions, capillary aneurysms of the retina may also be the source of hyaline bodies. Figure 12 shows a chain of four typical microaneurysms without hyalinization on a capillary of the clinically normal retina of a 67-year-old man who died of a myocardial infarct. His postmortem examination revealed an advanced, general arteriosclerosis. He had no diabetes and there was no central venous thrombosis of the eyes. In both eyes there were many retinal microaneurysms which were composed of a layer of hypertrophic endothelial cells surrounded by another layer of thick, circularly arranged connective tissue fibers.

Figure 13 demonstrates the completely hyalinized microaneurysms in the retina of a patient with Kimmelstiel-Wilson syndrome. This eye had developed an advanced diabetic retinopathy and hemorrhagic glaucoma secondary to central venous thrombosis. In this case, the microaneurysms represent homogeneous hyaline bodies; the elements of the wall can no longer be recognized and there is no lumen left. In other areas of the same retina, the capillaries, between the microaneurysms, have more or less disappeared and some microaneurysms remain in the tissue as single, round hyaline bodies (fig. 14). There are also areas where the whole capillary network together with the microaneurysms have become hyalinized (fig. 15).

The small punctate hemorrhages (fig. 16)

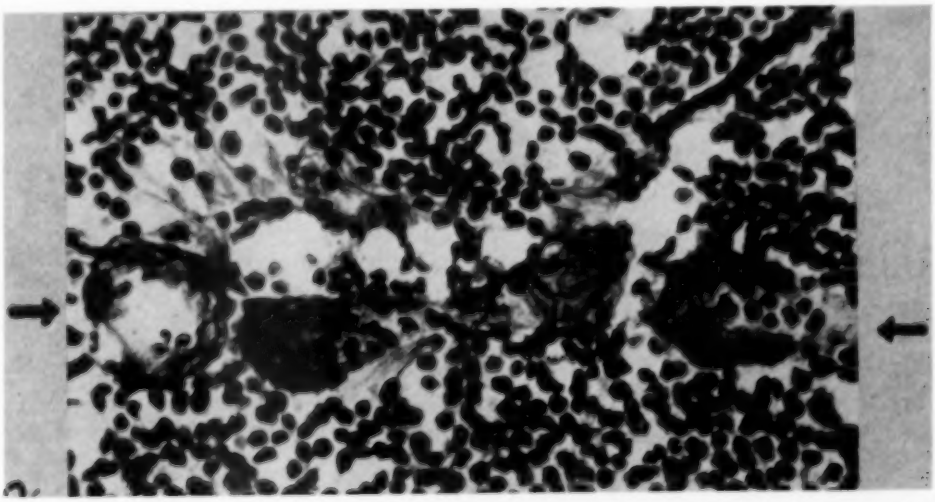

Fig. 12 (Wolter). Capillary with a chain of microaneurysms (between arrows) in the inner nuclear layer of an eye of a 67-year-old man who died of a myocardial infarct. The walls of the microaneurysms are composed of hypertrophic endothelial cells and a layer of thick connective tissue. (Hortega method, photomicrograph, ×300.)

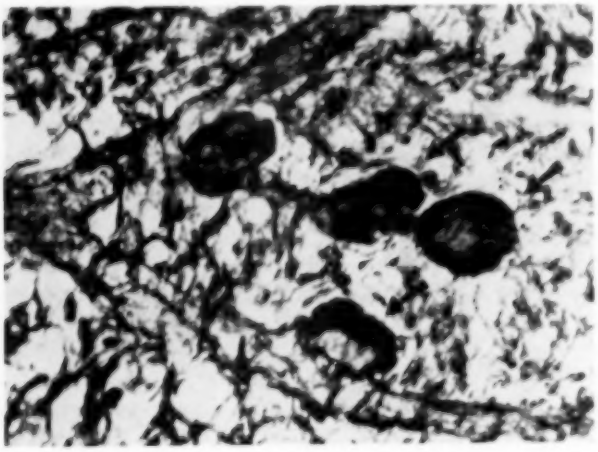

Fig. 13 (Wolter). Completely hyalinized microaneurysms (arrows) of capillaries of the retina of a patient with Kimmelstiel-Wilson syndrome and central venous thrombosis. (Hortega method, photomicrograph, ×300.)

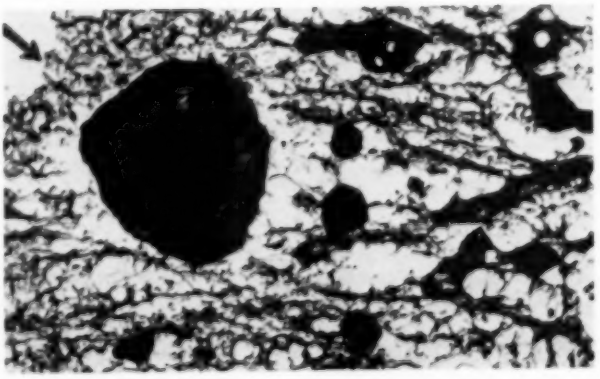

Fig. 14 (Wolter). Hyaline bodies of the same retina of the patient with Kimmelstiel-Wilson syndrome and central venous thrombosis which developed from capillaries and microaneurysms (arrow). (Hortega method, photomicrograph, ×300.)

Fig. 15 (Wolter). An area of the capillary network of the retina with a microaneurysm (arrow), both of which are completely hyalinized. Section from the eye of the same patient with Kimmelstiel-Wilson syndrome and central venous thrombosis (compare Figures 13 and 14). (Hortega method, photomicrograph, ×300.)

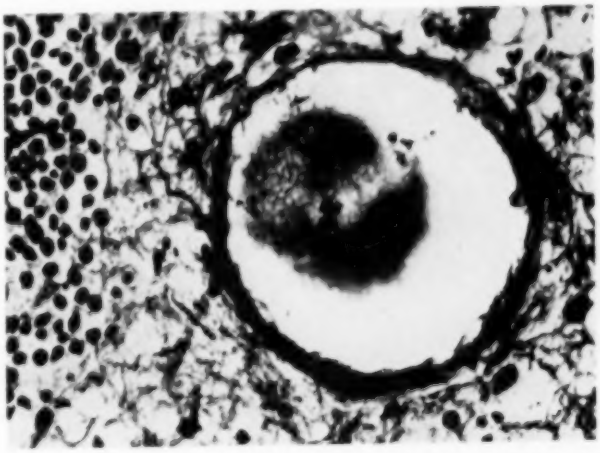

Fig. 16 (Wolter). Punctate hemorrhage of the retina of the patient with Kimmelstiel-Wilson syndrome and central venous thrombosis. The remnants of blood and the wall of glial fibers are clearly visible. (Hortega method, photomicrograph, ×350.)

which are also typical of diabetic retinopathy are always distinctly different from the microaneurysms and never show hyalinization. They represent cystlike formations with a wall of glial fibers, filled with blood in different stages of resorption.

We believe that the combination of the two disorders, Kimmelstiel-Wilson syndrome and central venous thrombosis, in both of which microaneurysms of the retina are known to develop (Friedenwald,[9] Ashton,[10] Becker and Post[11]) contributed to these especially severe changes in the present case. As to the case with typical microaneurysms of the retina without diabetes or central venous thrombosis of the eye, it should be mentioned that Hartford[13] has already described the occurrence of microaneurysms in clinically normal eyes.

The hyalinization of pigment epithelium cells of the retina is a very common occurrence in senescence. It also may occur in chronic eye diseases. Figures 17 and 18 are taken from sections of the clinically normal eye of a 96-year-old man.

Figure 17 clearly shows the beginning of hyaline degeneration of a cell of the pigment epithelium. The nucleus and some pigment granula are still visible.

Figure 18 shows more advanced stages of this hyalinization. Here the nuclei are only very faintly visible and all cell structures are replaced by hyaline substance.

The deposition of hyaline substance on

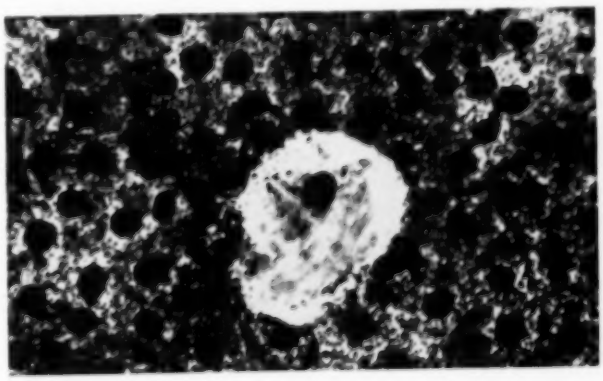

Fig. 17 (Wolter). Horizontal section through the pigment epithelium of the eye of a 96-year-old man. One cell shows degeneration and beginning intracellular accumulation of hyaline. (Hortega method, photomicrograph, ×800.)

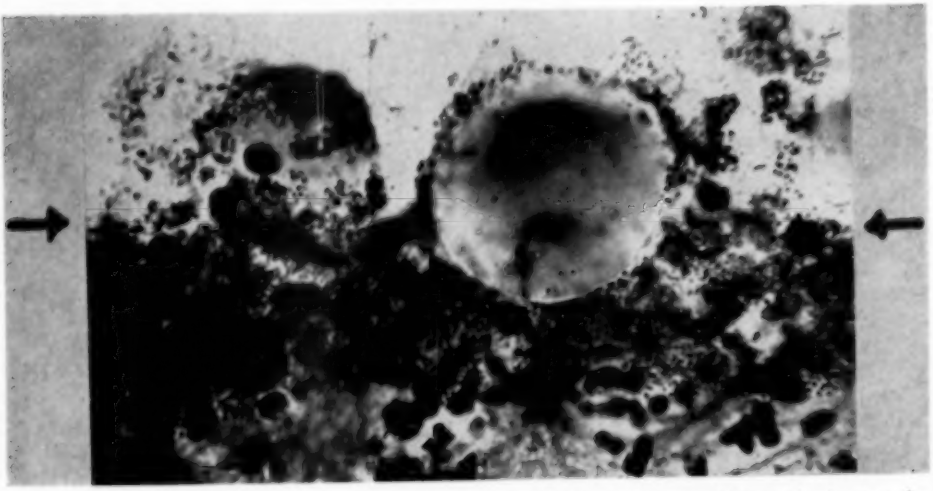

Fig. 18 (Wolter). Two more advanced stages of accumulation of hyaline substance in degenerating cells of the pigmented epithelium (arrows) of a 96-year-old man. In both cells, the nucleus and some pigment granula are still visible. (Hortega method, photomicrograph, ×800.)

Bruch's membrane beneath the pigment epithelium cells appears to be the more common type of hyalinization in this layer (compare Rones[15]). Both previously described types of hyalinization: (1) The accumulation of hyaline substance within the pigmented cells, and (2) beneath the cells, may occur one beside the other in the same eye.

Many theories have been offered to explain the nature and origin of these "colloid bodies," "hyaline bodies," or "drusen" of the pigmented epithelium layer. These theories have been divided by Coats[14] into two groups: the transformation theories and the deposition theories. The present observations suggest that both theories are right. Deposition of hyaline substance may occur beneath the intact pigment epithelium as well as in the cells of this layer. Large drusen of the same shape and arrangement, which finally take the space of the pigmented cells, may develop from both types of hyalinization.

It was generally believed that the formation of drusen is due to local disturbance of blood supply. It is interesting to note, however, that not infrequently large drusen may develop on one side of Bruch's membrane, while on the other side there are normal-appearing, thin-walled capillaries, filled with blood, and directly attached to the membrane. This is rather difficult to interpret.

Hyalinization of retinal elements may also be observed in the typical glistening white spots which are seen, for example, in a "star figure" of the macular area. Figure 19 shows a section of the retina of a patient with arteriospastic retinopathy (albuminuric or nephritic retinitis).

Typical gittercells which are loaded with lipoids from broken-down neurons are seen piled up in some areas of the inner retina (19-A). For an unknown reason, these gittercells are apparently unable to carry the lipoids to the blood vessels, as they do normally, but remain in the retina and finally become necrotic. In Figures 19-B and 20-A and B, different stages of this necrosis of the gittercells and hyalinization of the whole remaining substance are visible. The end results of this process are permanent depositions of hyaline substance which are mostly

arranged in a radiating manner in Henle's fiber layer. These depositions must be considered to be the histologic equivalent of the white structures of the star figure.

Exactly the same histologic findings as in this case of arteriospastic retinopathy were demonstrated in both eyes of a patient who exhibited a star-shaped figure at the macular area in advanced diabetic retinopathy.

It must be emphasized that the phagocytes which carry the substance of broken-down neurons, pile up and finally become hyalinized in this condition, represent gittercells (also called granular compound corpuscles). Gittercells are swollen microglial elements which have phagocytized lipoid substance. These cells as well as their origin and function are very well known from anatomic and pathologic studies of the brain and the optic nerve. They are of mesodermal origin and may be compared with the histiocytes of the mesodermal tissues. This is contrary to the opinion of Leber who believed that these phagocytes are derived from the pigment epithelium of the retina.

An outstanding finding in this study was the complete hyalinization of the choriocapillaris. This was observed in both eyes of a patient with advanced retinitis pigmentosa. Figure 21-B represents the completely hyal-

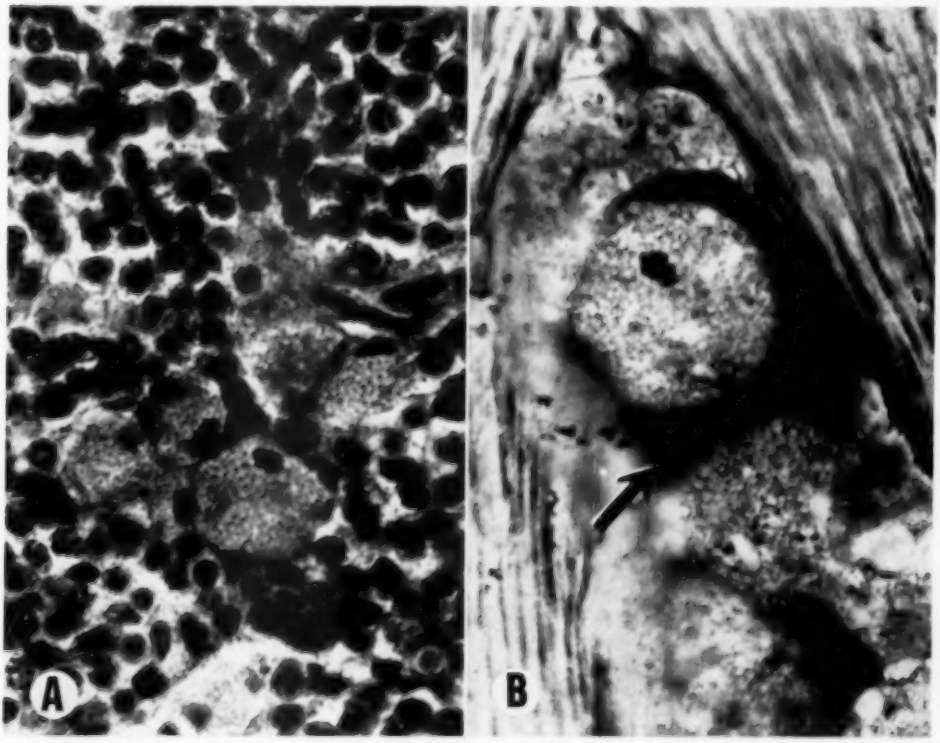

Fig. 19 (Wolter). (A) Phagocytes (gittercells) which are piled up in the ganglion-cell layer of the central retina of a patient with arteriospastic retinopathy.

(B) Necrosis and beginning hyalinization (arrow) of gittercells in Henle's fiber layer of the retina of the same patient with arteriospastic retinopathy. The lipoids within the gittercells and in the necrotic areas are extracted by the histologic procedure and represent empty vacuoles. (Hortega method, photomicrographs, A: ×600; B: ×900.)

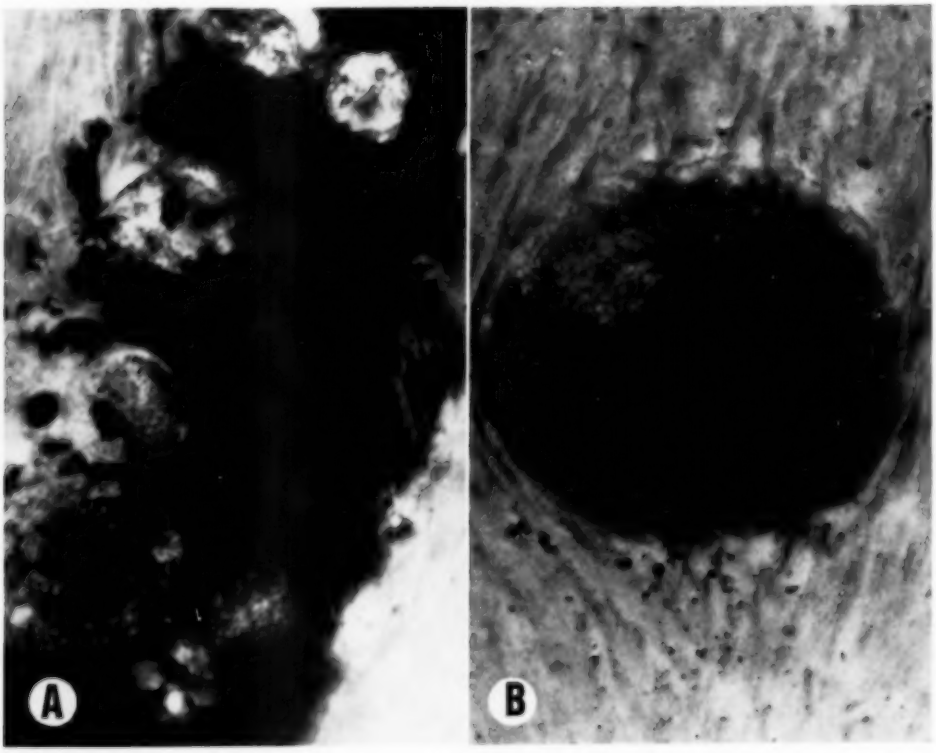

Fig. 20

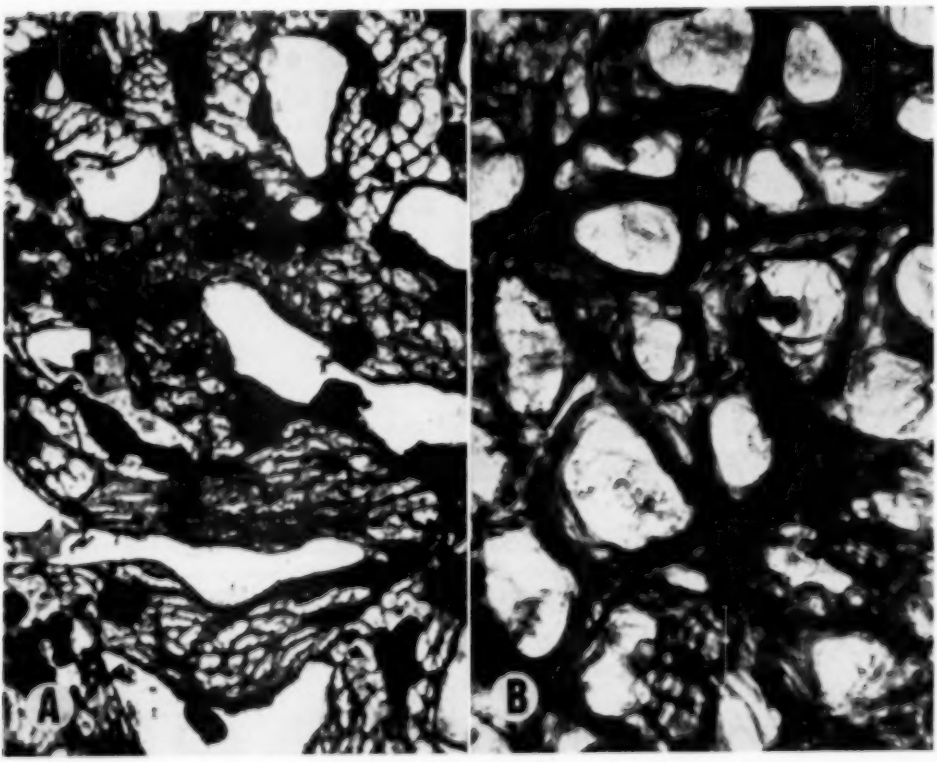

Fig. 21

Fig. 20 (Wolter). (A) Advanced hyalinization of broken-down gittercells in Henle's fiber layer of the retina of the same patient with arteriospastic retinopathy (compare Figure 19). Some normal gittercells are visible in the hyaline substance.

(B) Large hyaline body in Henle's fiber layer of the retina of the same patient with arteriospastic retinopathy. In the left upper area in the photograph, there are still the remnants of a necrotic gittercell visible. (Hortega method, photomicrographs, ×700.)

inized choriocapillaris in this case. The tissues of the capillaries can no longer be recognized and there is no lumen left.

This finding is interesting in comparison to many other cases of retinitis pigmentosa which were described to have no changes of the choriocapillaris or choroid. There is a recent report, however, which also describes vascular changes in the choroid of a case of this disease (Lucas[16]). Figure 21-A shows a flat section of a normal choriocapillaris for comparison.

3. Pathology of the visual cells

Changes of the neuroepithelial layer of the retina, under pathologic conditions, are too complex to be discussed here. It may be mentioned, however, that they show histologically nonspecific reactions which are basically the same as those described above for the neurons of the ganglion-cell layer.

In acute involvement of the retina there is sudden breakdown of the neurons of the outer layers. In chronic conditions there occur slowly progressive, degenerative changes of these cells. They usually begin with the outer processes of the rods and cones, later involve the inner processes, and end with degenerative changes and the final destruction of the cell bodies. Naked nuclei of a pyknotic condition may remain for a while after the destruction of the cell bodies and their processes. The debris of the destroyed cells are phagocytized and removed by microglia elements (gittercells).

Figure 22 is an example of degeneration of the neurons of the outer layers of the retina. It is taken from a section of an eye of a patient with old retinal detachment. There is severe deformation, swelling, and beginning dissolution of the visual cells. In most of these cells, the outer and inner processes show the severest damage. Others have disappeared completely leaving cystlike spaces (a) in the outer layers.

4. Typical pathologic changes of the nerve fibers of the human optic nerve

The nerve fibers of the optic nerve are mainly composed of neurites of the neurons of the ganglion cell layer of the retina. Some fibers, however, originate in the brain and end in the retina (efferent, antidrome fiber, Wolter and Liss[17]). The pathology of all fibers of the optic nerve is very similar to that of the fibers of the nerve-fiber layer of the retina, which was described above.

There are two definitely different types of nerve-fiber degeneration in many diseases which involve the optic nerve. The first kind occurs in acute situations and represents a sudden fragmentation resulting in the destruction of the nerve fibers. Figure 23 shows, as an example, the acute destruction of all nerve fibers in a case of direct compression of the optic nerve by a fast-growing craniopharyngioma. All nerve fibers are literally broken into pieces and represent small fragments which already begin to disappear.

The other typical form of degeneration is the slowly progressive degeneration which occurs in chronic diseases. Interruption of

Fig. 21 (Wolter). (A) Horizontal section of the choriocapillaris of a normal eye.

(B) Completely hyalinized choriocapillaris of the eye of a patient with an advanced retinitis pigmentosa. (Hortega method, photomicrographs, ×700.)

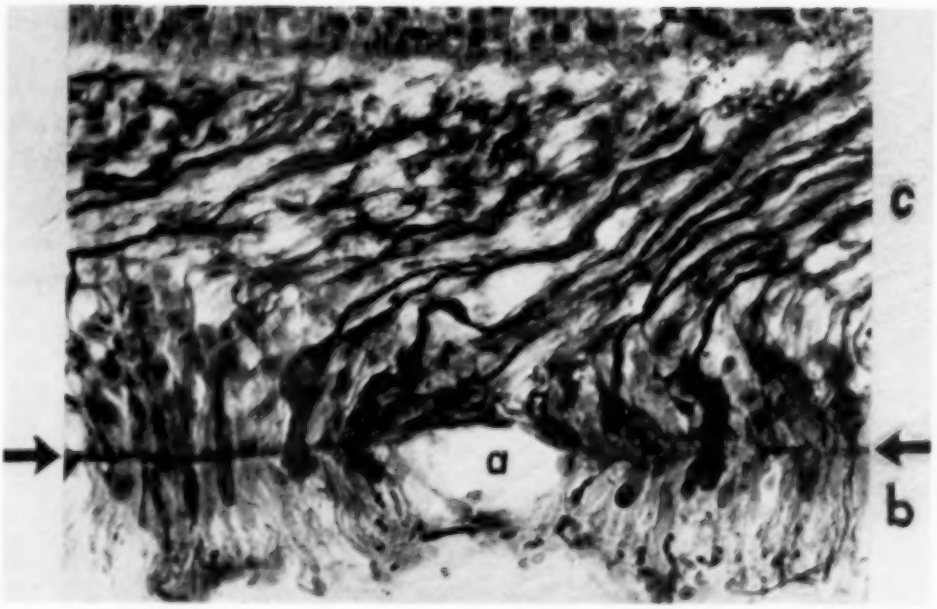

Fig. 22 (Wolter). Outer layers of the retina of a case of old retinal detachment. Arrows indicate the outer limiting membrane. (a) Cystlike space of destroyed neurons. (c) Degenerated inner processes of visual cells. (b) Degenerated rods and cones. (Hortega method, photomicrograph, ×700.)

the fibers and development of bizarre terminal swellings of the distal stumps in retrograde degeneration are typical signs of this process. Figure 24 illustrates this type with interruption and typical terminal swelling of a nerve fiber of an optic nerve which was chronically compressed by a slowly growing meningioma.

A third type of nerve-fiber degeneration is often seen in single fibers of the optic

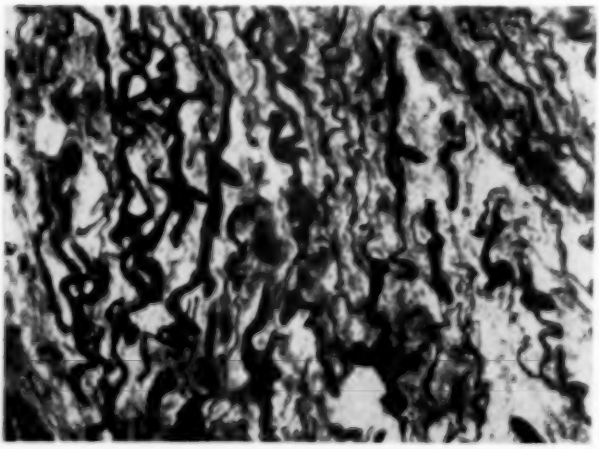

Fig. 23 (Wolter). Acute fragmentation of nerve fibers of the optic nerve in a case of craniopharyngioma. (Hortega method, photomicrograph, ×500.)

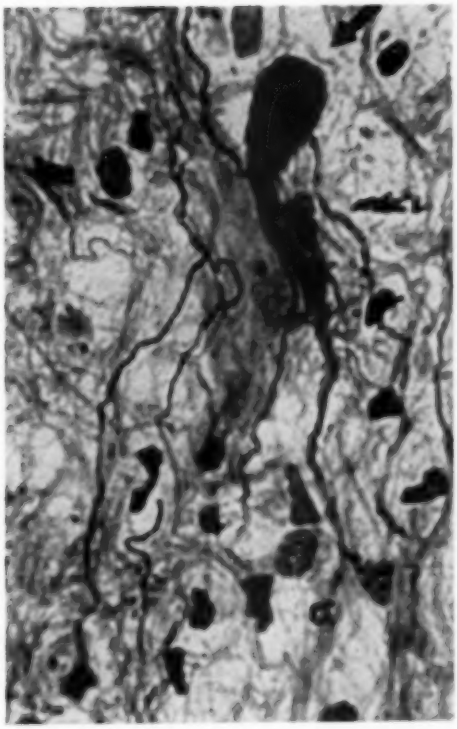

Fig. 24 (Wolter). Terminal swelling of a nerve fiber in slowly developing retrograde degeneration (arrow). Section from the optic nerve of a case of meningioma which chronically compressed the optic nerve. (Hortega method, photomicrograph, ×500.)

Fig. 25 (Wolter). Hyaline body within a nerve fiber of the optic nerve of a normal senescent eye. (a-a) Nerve fiber. (Hortega method, photomicrograph, ×500.)

nerves of senescent eyes. Figure 25 represents this type of degeneration with the development of the same round hyaline bodies in the nerve fibers as we described it in the nerve-fiber layer of the retina. Very often these hyaline bodies appear alone in the tissue without connection to the nerve fibers. In such cases it is not possible to decide whether the hyaline bodies have developed from nerve fibers or from astrocytes. Degeneration of astrocytes of the optic nerve also may result in very similar hyaline bodies.

This demonstration should be interpreted as an effort to describe and classify a few of the numerous cellular reactions of the retina in common morbid entities. It is not at all complete in literature, description, and discussion, and stirs up many more new problems than it solves. It is our belief that such studies, as this one, concerned with the basic details of eye pathology, are helpful for the understanding and the treatment of disorders of the eye.

University Hospital.

ACKNOWLEDGMENT

It is a pleasure to express sincere thanks to Dr. Fralick, Dr. Falls, and Dr. Henderson of the Department of Ophthalmology of the University of Michigan Hospital whose encouragement and help in obtaining pathologic eyes made this study possible.

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Discussion

Dr. Parker Heath (Sullivan Harbor, Maine): A good title for Dr. Wolter's paper would be "Some of the degenerative changes within the retina and optic nerve from chronic processes as shown by silver-carbonate staining." For an extra fillip the author has included a discussion upon hyaline-colloid formations and a note on some degenerative changes in the rod-cone layer.

The over-all processes described are either those of late stages, and long acting, or the results from severe injury. The author has indicated these boundaries within the paper.

The usual conception of late-stage retinal neuron pathology is that of similar reactions to different injurious agencies. The degenerative patterns of slowly acting injuries differ in degree but are comparatively nonspecific. Inflammatory cells are not conspicious.

The injury fragments or destroys the dendrites and neurites. The neuron undergoes degenerative changes—a matter of degree—from swelling, granularity, vacuolization, loss of outline and staining capacity, cytoplasmic and nuclear extrusions, and so on, to complete wreckage. Gittercell phagocytosis of lipid substances and glial cell scarrings are typical.

The silver-carbonate method beautifully exploits these changes and their sequences. Let us remind ourselves that the retina although transparently thin is capable of isolated lamellar reactivity. Early stage reactions can be selective and limited to small groups of elements, that is, isolated colonies of rods or cones or cells of the pigment and ganglion layers. Experimental material is usually acquired easily but that from humans is slowly built up but each tends to complement the other. We hope that early stages of various kinds of retinal pathology and the minor and sometimes reversible injuries will be subjects for future reports by the author.

Now to discuss what all of us are looking for—the new and the authentic from his presentation. The bulblike terminal swellings of the nerve-fiber layer from old-stage retinal detachment have been described before but their conversions to a globular lipid degenerative biproduct is only now clearly shown. And the apparent attempt toward regeneration of the distal end of an interrupted nerve fiber, the axon, is a new concept. The bizarre coiled formation is clearly seen in the author's preparations and may represent an abortive budding regeneration.

The various hyaline formations are of especial

interest. Found in the nerve fiber layer are globular bodies, representing a nonspecific kind of hyaline degeneration of the nerve fibers—these are often called cytoid bodies. Ganglion cells also may degenerate into cytoid hyaline spheroids. I agree with Dr. Wolter as to the origin and significance of these bodies. I furthermore include in the cytoid body group, hyaline degenerated astrocytes, and hyalinized microaneurysms. The latter are easily found, as pointed out by the author, in properly prepared material from postvascular occlusions, or from hypertensive retinopathy, or from Kimmelstiel-Wilson syndrome and even material with normal clinical history.

The pigment epithelium hyaline changes described by the author are in accord with my conception of the role of this tissue; namely, that Coats was right as to the two theories of origin: (1) from pigment epithelium by intracellular reaction and (2) deposition under the pigmented epithelium probably from vascular origins. Also drusen may develop in large proportions irrespective of recognizable choriocapillary changes.

The glistening spots in the star figures of the macula may well be gitterceils in depôts—stored and immobile collections of lipids within mesodermal phagocytes.

The described hyalinization of the choriocapillaris from a case of retinitis pigmentosa probably represents a secondary process not seen during early stages.

The pathologic changes of the rod and cone elements which the author reports resemble those found in the inner layer. This is true of chronic and late stage processes.

The classification into two groups of the pathologic changes within the optic nerve, namely, acute, severe with marked fragmentation and destruction, and slowly progressive with bizarre terminal swellings of retrograde degeneration, is satisfactory as far as it goes. It would seem that the author did not mean this to be an all-encompassing classification but instead a working one subject to added chapters.

Many of the processes described by the author are only visible when the silver carbonate technique is used. Dr. Wolter is doing pioneer work which has already made a substantial contribution.

A STUDY OF INTRAOCULAR Vx2 AND ITS TREATMENT*

ADOLPH W. VOGEL, M.D., AND KHALIDA NAIB, M.D.
Philadelphia, Pennsylvania

A wish invariably voiced by the ophthalmologist encountering a small intraocular neoplasm is that there could be available a repetitively successful method to destroy it, with retention of a functional eye. Until this problem is solved, an experimental eye lesion is needed for the evaluation of new applicable therapeutic weapons and further trial of presently used methods.

A possible such tool is the Vx2 rabbit carcinoma[1] which can be readily transplanted into the rabbit fundus.[2] The following observations are presented to describe the reactions of the normal rabbit eye to its presence and to compare the effectiveness of several agents against it.

* From the Department of Research, Wills Eye Hospital, Irving H. Leopold, M.D., Director. This work was supported by Damon Runyon Fund, Inc., Grants #254 and 254A(T).

TRANSPLANTATION OF Vx2

STOCK

Stock tumor was maintained in five- to six-pound pigmented mongrel rabbits by intramuscular thigh transplantations every three to four weeks. The mass usually is first palpable as a small deep nodule two weeks after passage and will grow to a diameter of five cm. in four weeks. Intramuscular growth is apparent as a roughly spherical shape from the centrally located original transplant. As it enlarges the older center cells die and the viable tumor tissue forms a constantly enlarging shell of varying thickness surrounding a liquid cavity. This capsule of living tumor cells has a rather coarse granular texture, cuts with a gritty feeling, and is yellowish pink. Its inside surface facing the center is well de-

fined; the outside invading surface, poorly defined.

The histologic picture is one of a very poorly differentiated carcinoma. No clear hint as to its original squamous-cell origin was given in any of the sections examined by the formation of pearls, or even rudimentary keratin deposits. The cells are 25 to 30 μ in diameter, with most of the area occupied by a dark basophilic nucleus showing heavy chromatin clumping. The cytoplasm boundaries are well defined and round in relatively separated cells. In closely packed groups the cytoplasm often merges so that no definite margin between adjacent cells can be seen. Many mitotic figures are scattered throughout the tumor and not infrequently multipolar spindle formations can be seen.

Necrosis was met in all takes. The cell nuclei become small, intensive in staining, and irregular in shape. Later they fall apart and the cytoplasm is released as a pink-staining background sprinkled thickly with chromatin nuclear fragments. The most obvious and easily explainable cause of the necrosis seen even in the early stages of a take is rapid outstripping of an insufficient blood supply. The whole behavior of the transplant indicates this. Its vascular system is a primitive one, frequently not endothelial lined but more often a sinusoidal-like blood channel.

Occasionally the original focus of cells was apparently deposited intermuscularly and the compressed connective tissue of the fascial sheaths formed a more definite tumor boundary which could be freed by blunt dissection. The neoplastic tissue removed was cut into small two- to three-mm. pieces and then forced through a 40-mesh monel metal sieve. The heavy material scraped from the obverse side of the sieve was diluted with normal saline 3:1 by volume.

This suspension could be injected intramuscularly via a No. 21 needle. Muscle fibers and connective tissue included in the suspension occasionally caused blocking and, in order to obtain a less bulky suspension, intraperitoneal passage was tried. In this location nodules are found subperitoneally and consist of purely Vx2 cell clusters with the endothelial peritoneal covering. The amount of material recovered, however, is less than in the intramuscular location and not infrequently absence of tumor indicated an inadvertent intestinal injection.

This intraperitoneal site was abandoned in favor of the intramuscular which gave takes in 39/41 and spontaneous regression in 2/41. This high percentage of successful passages is similar to that found in other laboratories.[3] A possible age-immunity factor was diminished by keeping within a five to six pound weight range throughout stock and ocular transfers.[4]

Ocular

The heavy material used for the passage of stock tumor was diluted 4:1 by volume with normal saline and allowed to stand for five minutes in a test tube. The still cloudy supernatant fluid was transferred to another tube surrounded by ice cubes. Cell counts of samples prepared in this manner show roughly 2×10^6 cells/cc.; 0.15 cc. of this preparation is drawn into a tuberculin syringe with a No. 27 needle (bevel 0.5 mm.). Entrance into the proptosed eye is halfway between the equator and optic nerve and usually two mm. superior to the course of the anterior ciliary artery.

Under ophthalmoscopic guidance the shortened tip of the needle is passed across the posterior chamber to the inferior margin of the medullated fibers below the nerve head. Here the retina is thickest and the needle can be most easily nosed into the subretinal space. When the shortened bevel is completely covered, 0.01-0.015 cc. can be easily injected to form a half-moon-shaped bleb. The inferior margin is usually well defined, and spreads below onto the avascular retina.

It is not unusual for a long strand or cloud of Vx2 suspension to billow out around

the needle shaft through the retinal hole. Withdrawal of the needle shows usually a long strand tenaciously clinging to the needle point. Swift needle withdrawal does not lessen this, and may tend to dislodge the subretinal suspension. If the scleral entrance site and that through the retina are approximate, the fluid may escape and the bleb collapse. Unintentional seeding of the scleral wound occurred in 80 percent of the eyes injected transvitreally, giving rise to a choroidal-scleral nodule (see later).

An attempt was made to confine the tumor cell deposit to only one nidus by using a single minute solid fragment of tumor tissue loaded into the barrel of a blunt No. 24 needle. The fragment was pushed out of the needle into a subretinal pocket by a close fitting stylet in a manner analogous to the routine subcutaneous transplantations of tumor tissue. This method was not used because of the mechanical difficulty in maintaining the fragment in position, as it tended to stick to the stylet and follow it into the vitreous back to the initial scleral puncture. Silicon ointment and interposing agar plugs between the tissue and stylet do not prevent their mutual adherence.

Intraocular growth

Location

Each organ of the rabbit viscera has been shown to support the Vx2 carcinoma.[5] Ginder and Friedenwald[6] observed that suspensions of Vx2 disappeared completely from the anterior chamber and after a latent period of four or five days' growth appeared only from particles which had settled in the iris crypts. In this laboratory vitreous suspensions of varying size as a result of regurgitation along the needle shaft were observed in 120 of 254 eyes used, and vitreal solid fragments were suspended in the vitreous in 18 eyes. No growth from their margins or increase in size occurred in any of these deposits, and they gradually become more compact in appearance, sometimes breaking up into tiny globules resembling asteroid hyaloid bodies. The larger particles may remain visible for weeks, gradually contracting and finally disappearing leaving a vitreal haze and/or bands. Atrophy of the solid fragments is indicated by an ensuing dead white color. This indicates that the tumor requires more nourishment than the vitreous affords and that successful takes require actual host tissue contact.

Of 137 eyes studied pathologically, two distinct tumor sites were discernible in 105, either by ophthalmoscopic or postmortem examination. Takes occurred choroidal-scleral at the needle hole in 36 percent, below the nerve head in 19 percent, and as double foci in 45 percent. In the remaining 32 eyes, the origin of the existing tumor could not be determined. The choroidal-scleral nodule was located always in the same general area, and externally oriented as follows:

Four to six mm. anterior to the nerve head and 1.5 to 2.0 mm. superior to the anterior ciliary vessel (figs. 1 and 5). This position was repeated because the rabbit eye turns on its axis as it is proptosed and this area is presented as the most accessible for a transvitreal passage.

Ophthalmoscopic appearance of a take was noticed within 10 days in 48 percent, from 11 to 15 days in 40 percent, between 16 and 30 days in 12 percent. A take at either site is first noticed as an increased coiling and darkening of the overlying terminal end capillary loops.[7] This is caused by the small beginning solid detachment and consequent interference with the retinal nourishment. The involved areas are usually 0.25 disc diameters when first noticed. Gradual increase in size occurs with eventual total elevation of the anterior medullated wing if the two sites merge.

Ocular reaction to inert Vx2 material

Six eyes were injected subretinally with necrotic Vx2 tissue taken from the cavity of an intramuscular tumor. Thawing and refreezing four times at −20°C. was done to eliminate viable cells and the material stored

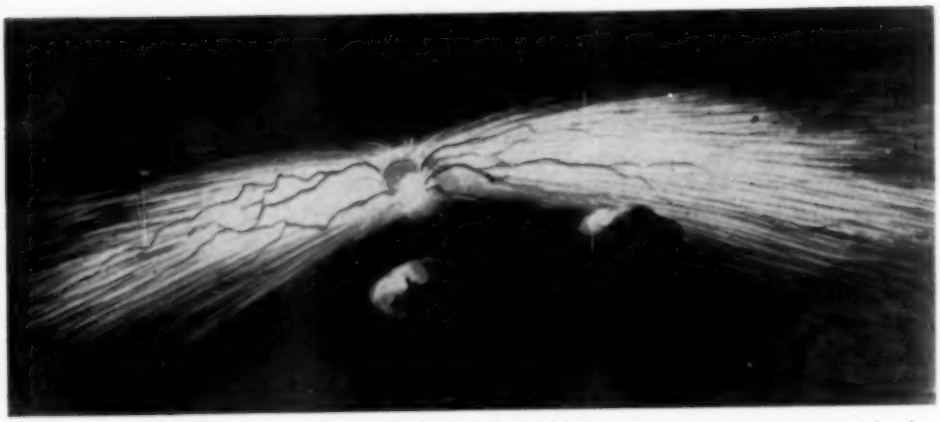

Fig. 1 (Vogel). Ophthalmoscopic view of double foci of V×2, one location below the nerve head, the second at the usual choroidal-scleral site slightly under the anterior wing.

at the same temperature for one week. The eyes were enucleated four days after injection. The anterior one half of all the eyes was normal.

Fundal changes were shown by glial retinal changes as found following the subretinal injection of normal saline in control eyes. A few chronic inflammatory cells were seen near the retinal vessels over the nerve head. The choroid showed slight vascular dilatation in three eyes but without any accompanying cellular response.

No absorption of the suspension had taken place. Several areas of beginning calcium formation were present in the subretinal deposits. The episclera was normal.

Ocular reaction to viable Vx2

The interaction between host and transplant in higher vertebrates as a rule begins within the second week, and is evidenced by cellular and stromal response.[8]

The ocular response to the presence of this highly anaplastic tissue was mostly confined to the uveal tract and episclera. Of 27 control eyes observed for a period between two to four weeks 66 percent showed a heavy episcleral chronic inflammatory cell infiltration and the rest displayed only a moderate to scattered infiltration. The choroid evidenced many easily seen small round cells, especially near the base of the tumor in 40 percent. This was accompanied by moderate engorgement of the choroidal intervascular space and beginning increase in vessel diameter. In four eyes many cells were seen near the tumor base and also scattered out to, but not including, the periphery. One eye showed closely packed cells throughout the entire choroidal area except the periphery (fig. 2). The remainder showed only a slight choroidal sprinkling of round cells.

Thirty-one control eyes were observed for

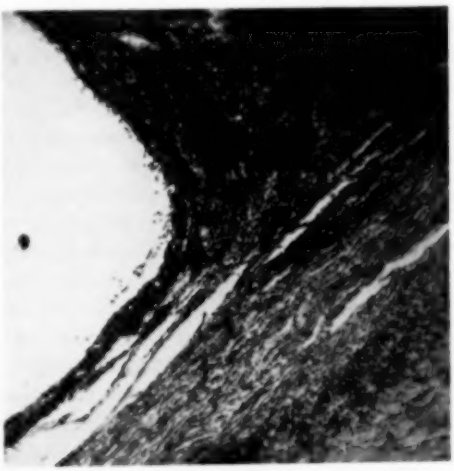

Fig. 2 (Vogel). Heavily infiltrated choroidal and episcleral tissue at base of Vx2 tumor.

a period between one and two months; 73 percent showed moderate to heavy episcleral accumulations of chronic inflammatory cells, and the rest a slight increase. The choroidal cellular reaction in this group was distributed in essentially the same manner as in those animals surviving for one month. The cells, however, stained less intensely and individual dense foci were less frequently seen.

Choroidal scarring in the entire control group ranged from beginning increase in fibroblasts and chromatophore migration in 70 percent to frank massive thickening of the choroidal stroma; clumping and proliferation of pigment cells of the underlying and adjacent tumor areas were found in 30 percent. In those eyes with a more intense stromal reaction there was not necessarily a corresponding increase in the cellular reaction. The wide degrees of cellular and vascular response often present in the same animal indicate that these host responses are not necessarily interdependent.

Retina

All control eyes showed varying degrees of serous and solid detachment. Figure 3 shows a small clump of cells in an eye removed 12 days after subretinal passage and four days after the first ophthalmoscopic signs of a take. At the time of enucleation the 0.25 disc diameter area below the nerve head was showing a beginning loss of transparency and questionable flat elevation. There is loss of the rod and cone layer in the immediate vicinity. A small amount of subretinal pink-staining exudate containing several retinal nuclei and a few migrating pigment epithelial cells surrounds the neoplasm.

At this early stage there is little evidence of reaction between host and transplant. Scattered small round cells appear in the episclera overlying the tumor area, and the choroid is slightly engorged with a sprinkling of lymphocytes in the immediate underlying and adjacent vicinity.

All control eyes with takes showed some degree of solid and serous detachment. Sixty percent of 62 eyes examined had total solid and serous retinal detachment. In contrast only 11 had detachment confined to the tumor summit. The rest displayed various degrees of solid plus serous detachment from areas

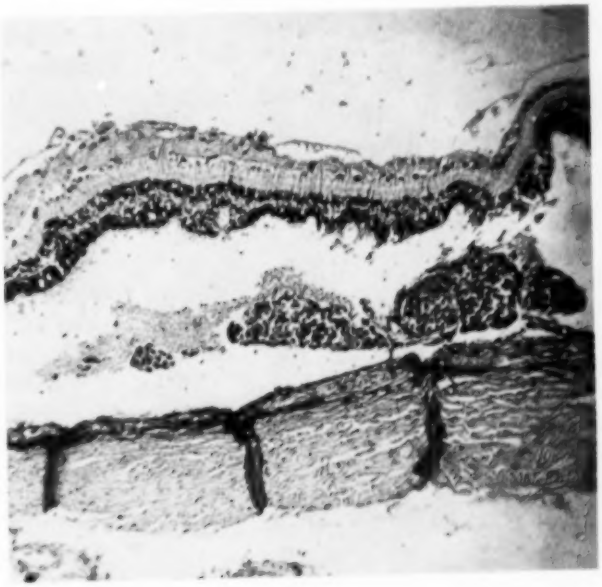

Fig. 3 (Vogel). Beginning subretinal Vx2 tumor with minimal damage of surrounding structures.

confined to the immediate tumor vicinity tip to total detachment. The extent of the choroidal serous outpouring was not correlatable with the host cellular and stromal response. Inferior detachments usually connected directly to the tumor, but often an apparent unconnected peripheral pooling by gravity appeared first followed by adjacent posterior and anterior billowings.

Slightly over half of the eyes with total solid and serous detachments were observed for a period of between 30 and 60 days, while only one in this group was observed for less than two weeks. The rest survived for two to four weeks. In 25 eyes with detachment varying from mechanical displacement by the tumor only to partial serous and solid detachment, survival time varied from less than two weeks to greater than 60 days. An increase in permeability of the choriocapillaris is indicated in earlier ophthalmoscopic views by the occurrence of choroidal hyperemia and occasional petechial hemorrhages near the border of a solid detachment. The lifting up of the retina by serum obscures this in most eyes, and it was therefore observed particularly in those eyes in which the solid tumor was slow in enlarging. Five cc. of 10-percent fluorescein injected intravenously shows immediate passage into the vitreous from the vessels bordering the tumor base.

The absence of frank subretinal or vitreous hemorrhage indicates that as the choroidal vasculature is encroached upon there is concomitant occlusion of the vessel lumen. Figure 4 shows a choroidal vessel lumen filled solidly with tumor cells. The endothelium of the adjacent touching vessel is intact. Flat sections of the choroid failed to demonstrate any definite erosive or invasive action of Vx2 cells even in close proximity to the endothelial cells. Williams[9] has shown that this neoplasm has little or no ability to stimulate endothelial growth and that it depends upon the vessels already present in the region being invaded for its nutrition.

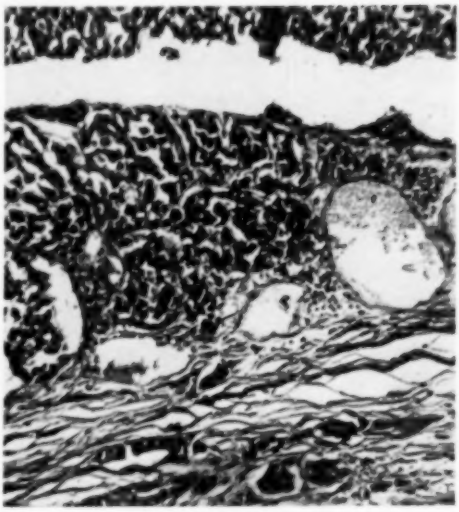

Fig. 4 (Vogel). Choroidal invasion by Vx2 with plugging of choroidal vessels. Beginning questionable erosion of adjacent endothelium.

Treatment

The applicability of this carcinoma as a standard lesion against which various agents can be used was tested with: the Stallard disc alone, and in combination with cautery; cautery alone and in combination with chemotherapy; chemotherapy alone. Of 69 animals treated unilaterally, seven showed a nonmalignant control eye, and these are not included in this discussion. The same surgical approach and postoperative treatment were used in all cases. Animals were selected if possible which had approximately equal bilateral neoplastic fundal involvement.

Anesthesia was given by Nembutal 0.2 cc./lb. intravenously, supplemented by open drop ether, pontocaine, and Novocain.

The conjunctival incision was started in the anterior cul-de-sac between the nictitating membrane and eyeball and carried superiorly and inferiorly 90 degrees. The entire anterior scleral one half from limbus to optic nerve can be visualized by retraction of the relatively rudimentary extraocular muscles. The vortex veins and path of the ciliary artery

are easily seen. This inspection which was carried out on all operated eyes frequently revealed a choroidal-scleral tumor (fig. 5). No. 4-0 black silk suture was used in interrupted conjunctival and lid sutures. The latter were used to decrease the amount of cage dirt introduced into the eye during the recovery period. Erythromycin and atropine ointments and penicillin 100,000 units, intramuscular, were used every other day for one week.

Stallard disc

The 10 and 5.0-mm. active diameter discs loaded with 1.0 mg. and 2.7 mg. of radium respectively, and molded to fit as closely as possible the radius of curvature of the posterior half of the rabbit sclera, were used.*[10] The lug-clip as described by Stallard was discarded because it was found that it was possible to place the disc accurately and maintain its position with greater ease by taking a 1.5-mm. scleral bite with each needle of a double arm 4-0 black silk suture (fig. 5). The disc was securely held in the loop by thin celloidin applied with a fine point brush (fig. 6). There was little or no reaction to the thin coating of celloidin used.

Stallard disc and cautery combined

Three animals were treated in this manner.

1. A choroidal-scleral tumor 4.0 mm. in diameter was cauterized (below) and then covered by the large Stallard disc for one week (3,500 r).

Sections made 34 days later show advanced glial changes of the retina underlying the disc, moderate choroidal fibrosis, pigment migration, and minimal cellular infiltration. No identifiable neoplastic debris could be seen. The adjacent retina showed loss of rod and cone layer and beginning merging of the nuclear layers. The retina away from the disc area and the anterior half of the eye were quiet and normal in appearance.

2. The anterior two thirds of a tumor involving an oval area 14 by 6.0 mm. was cauterized and the posterior one third which encroached upon the optic nerve was covered as much as possible by the Stallard disc for eight days (4,000 r).

Sections made eight days later show total serous and solid detachment with all stages of disintegration of the retinal elements, from early loss of the rod and cone layers to remnants recognizable only through the remaining characteristic debris pattern. Vacuolated, enlarged, and occasionally clumped tumor cells, many with abnormal mitotic figures or nuclear debris, were scattered in the vitreous and subretinal fluid. The area directly under the Stallard disc showed fragmented tumor only, while cells similar to those present subretinally were present in the cauterized area. The periphery of the cauterized area contained several deep islands of normal Vx2 cells surrounded by extensive areas of necrosis. The optic nerve area next to the disc edge showed strands of viable Vx2 cells.

3. A 1.5-mm. diameter tumor below the nerve head and a two-mm. diameter take at the needle site were cauterized. A scleral hole 1.0 by 2.0 mm. occurred at the intraocular site, below the nerve head. The small Stallard disc was applied so that it covered the cauterized needle site and half of the second focus below the nerve head. The scleral hole was not included. During removal of the disc seven days later (3,500 r) the animal died an anesthetic death.

Sections show the typical plastic appearance of the sclera in the cautery sites, marked choroidal vascular engorgement, and beginning pigment migration. No visible malignant cells are seen at the scleral nodule. The scleral perforation shows bordering cellular debris with glial changes in the overlying retina and several scattered overlying clumps of coagulated Vx2 cells.

* Radium Chemical Company, Inc., 161 East 42nd Street, New York 17, New York.

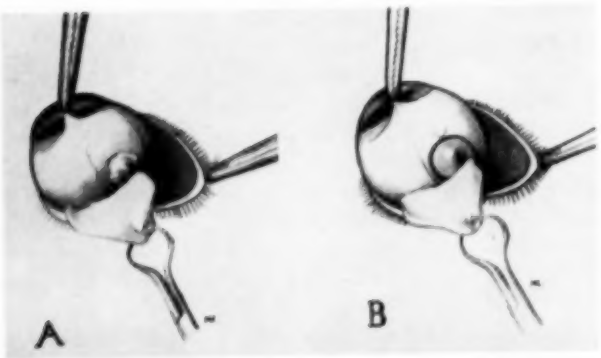

Fig. 5 (Vogel). (A) Showing surgical approach with a prominent choroidal-scleral nodule. (B) Stallard disc placed over tumor.

Figure 7 shows that a large globular mass of Vx2 had arisen during the time of Stallard disc application and was not histologically affected by either cautery or the adjoining radiation. Here the heat of the cautery was of enough intensity to produce a scleral hole yet dissipation by surrounding tissues and the choroidal circulation protected the tumor cells in an adjacent area.

Large Stallard disc

Seven eyes with tumors ranging in size from 2.0 to 6.0 mm. in diameter were treated in this manner. All eyes were non-malignant at the time of death. If takes at both the needle-site and below the nerve head were identified at operation the disc was placed to encompass both foci; a single take at the needle-site was noted in three, in one a take occurred only below the nerve head, and in two there was a double take. In six eyes the disc was placed within one week after the ophthalmoscopic discovery of a growth. In one animal operation was performed after a lapse of 13 days.

The observation time between removal of disc and death was less than a week in three animals (two, three, and six days). The control eyes all showed typical neoplastic cell formations with heavy cellular infiltration, choroidal and ciliary body engorgement, and complete and solid serous detachment. The treated eyes, in contrast, revealed foci of cellular debris marking the site of formerly viable carcinoma. Small clumps or strands of pale-staining ghost cells of Vx2 were seen on the periphery of these foci. The

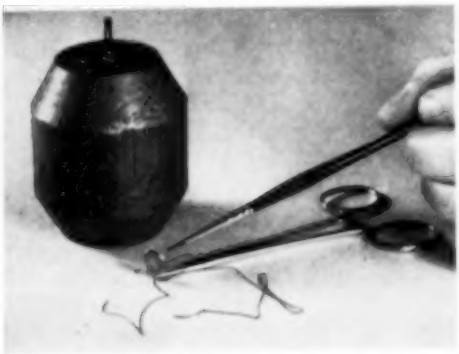

Fig. 6 (Vogel). Stallard disc, showing application of a thin coat of celloidin to hold a 4-0 double-arm black-silk suture in position.

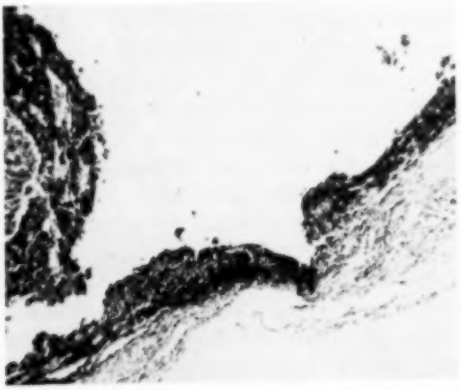

Fig. 7 (Vogel). Cautery area with scleral hole and adjacent vitreal mass of viable Vx2.

treated eyes presented a "drier" appearance than the control; there was less turgidity of the intraocular structures adjacent to the involved areas, the retinal architecture especially in the periphery was preserved, ciliary body engorgement was minimal, and cellular infiltration was chiefly in the immediate vicinity of the treated area.

Observation time after disc removal was less than two weeks (nine, nine and 11 days) in three animals. These eyes again showed retention of structural integrity of the uninvolved retina, and less cellular reaction and less engorgement of the bordering intraocular structures, than displayed by eyes examined within one week of therapy.

One animal was kept for 21 days after removal of the Stallard disc. Sections showed minimal small round cell infiltration, moderate gliosis of the retina with loss of the rod and cone layer, mergence of the inner and outer nuclear layers, and beginning pigmentary migration. A few ghost Vx2 cells could be seen, showing the marked irradiation damage with increase in cytoplasm and nuclear volume, vacuolization and faint staining qualities, which occurs before dissolution (fig. 8). The control eye presented massive round cell choroidal infiltration up to the ora serrata, massive retinal edema, and a serous hemorrhagic detachment containing many migrating pigment epithelial cells.

Small Stallard disc

Seven animals were treated in this manner. Of these four were nonmalignant at time of death. In one animal the anterior one fourth of the tumor could not be covered by the disc. After one week's coverage of the posterior three fourths, the disc was shifted from the original position to the previously uncovered area and left in place four more days (2,000 r). The time interval between removal of the disc from its second position to death of the animal was four days.

The intraocular changes again were

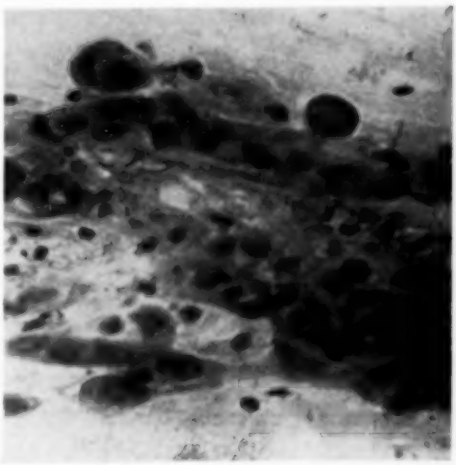

Fig. 8 (Vogel). A clump of dead and dying Vx2 shortly after removal of Stallard disc.

largely confined to the tumor area and the immediate vicinity. Beginning glial changes and scarring of the choroid are seen, and this area is strewn with numerous Vx2 cells undergoing various stages of dissolution. Giant cells are scattered in the surrounding area. The rest of the intraocular structures are normal.

One application of the small Stallard disc was used in six eyes. Of these, three were nonmalignant at the time of death. Times of observation after treatment were seven, 21, and 28 days.

In the animal killed one week after disc removal, sections showed that the neoplastic focus had been changed to a dark staining aggregation of necrotic material which lacked any cellular definition. Many cells surround these areas and display the features of irradiation, that is, pale nuclei and cytoplasm, increase in overall cell size, increase in cytoplasmic and nuclear size, and diminution in cell outline. There was moderate atrophy and avascularity of the retina and choroid with slight similar reaction in the immediately adjoining intraocular structures. Numerous giant cells lay on the area outskirts. Two eyes observed for three and four weeks show a quieter picture with rare giant

cells surrounding neoplastic debris which had broken so finely as to be almost amorphous.

In contrast to the numerous cells seen in those animals killed shortly after therapy only occasional scattered faintly staining cells were picked up in the periphery of the lesion. The malignant eyes all showed typical Vx2 clumps particularly near the nerve head, and the usual area of cell destruction under the Stallard disc. These eyes were not "dry" in appearance and marked chronic inflammatory cell reaction was seen in two.

LARGE AND SMALL

Two animals were treated in this manner.

1. The 98 sq. mm. combined surface area of the two discs was applied to a large tumor with a surface area of approximately 110 square millimeters and left in situ one week (3,500 r). The interval between disc removal and death was one week. Slight increase in the tumor mass occurred during therapy and exophthalmos developed after removal. Sections showed complete solid and serous detachment, the typically engorged and infiltrated structures, and a large scleral nodule and intraocular mass, composed almost entirely of the necrotic broken bodies of poorly staining Vx2 cells. Scattered clumps of viable Vx2 were seen near the ora serrata and invading the ciliary body.

2. The large and small discs were applied respectively to a three-mm. diameter take at the needle site and a one-mm. diameter lesion four mm. below the nerve head, and left in situ for one week. Death occurred two days after disc removal. As in other eyes removed shortly after therapy there were numerous dying Vx2 cells preserving the general tumor architecture but no typical healthy Vx2 congregations could be seen.

COMMENT (fig. 9)

Of 20 eyes treated with the Stallard disc, 13 did not show any viable Vx2 tumor cells. One showed the characteristic general tumor pattern but this was formed by cells with definite signs of severe irradiation damage. Six animals showed typical malignancy of the treated eyes. In 11 eyes, to which the large Stallard disc was attached, definitely viable Vx2 was found in one. At the time

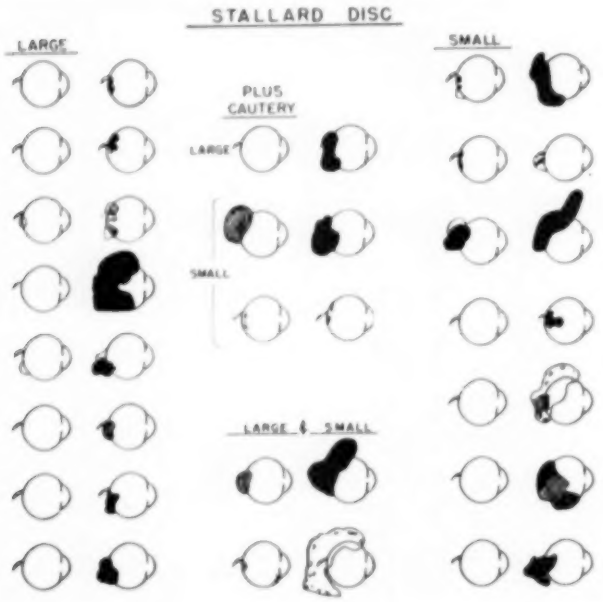

Fig 9 (Vogel). Outlines of cross section of eyes treated with Stallard disc. Solid black indicates typical viable Vx2; shaded area indicates recognizable but dying Vx2; empty areas indicate no viable Vx2 are present.

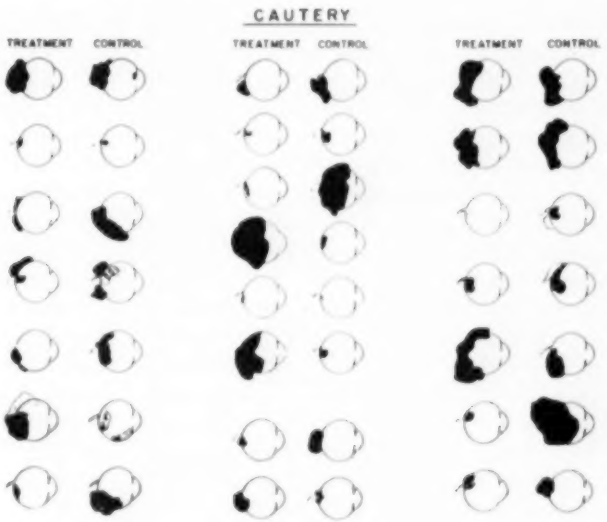

Fig. 10 (Vogel). Outline of cross sections of eyes treated with cautery. Markings are the same as in Figure 9.

this animal was operated, it was not felt that the margins of the two discs used formed a wide area of safety around the two tumor foci identified before and during operation.

The small disc was applied to nine eyes. Of these four were nonmalignant. Two of the malignant eyes had in addition been treated with cautery. The lower incidence of malignancy in eyes covered with 78 sq. mm. of disc area as compared to those in which 20 sq. mm. were used indicates that the close approximation of radiation areas to tumor areas permits, literally, a transection of visible or invisible extensions from the main mass, and their continued flourishing. It also suggests that unless the absolute tumor boundaries are known a generous disc margin of ionizing radiation is necessary.

Cautery

Twenty-two animals were treated in this manner (fig. 10). The same cautery machine* was used in all cases and the surgical technique was as already outlined.

The current used was 0.1 to 0.2 ma. The choroidal-scleral nodules which were easily identifiable on the scleral surface were attacked first under direct vision using a pointed electrode 0.5-mm. wide. Multiple applications of five- to 10-second counts each were made until the protruding surface was shriveled flush with the scleral surface. If the mass was more than one mm. deep, it was frequently necessary to remove eschar in order to accomplish this. In no instance was there any clearly recognizable regrowth from the surface of a nodule so treated.

The diathermy treatment was then extended for a 1.0 to 1.5-mm. rim around the base of the tumor. This surrounding area was also checked ophthalmoscopically and any extensions visible were also cauterized. A combined transilluminator-cautery was used for treatment of the purely intraocular[11] neoplasm located below the nerve head. The application was again surface with a 0.2 mm. electrode.

Perforating diathermy was not used as white coagulation of the overlying retina and parchmentlike reaction in the sclera were produced easily through the very thin rabbit sclera. It was expected that, with these changes, the layers between were also sufficiently altered. The strength of current used

* Vacuum-tube oscillator, Thermosector Company, 938 North Austin Boulevard, Oak Park, Illinois.

was not a criterion of damage as much as were the visible changes produced in the areas exposed surgically and those checked ophthalmoscopically.

The tumor area, defined as closely as possible beforehand, was watched by the light of the transilluminator as the current was applied. Connecting snow-white coagulated zones with immediate dispersal of the retinal pigment epithelium were first laid down encircling the tumor bed, then the central area was coagulated with repeated five- to 10-second applications until it too was completely white. Frequently the retinal vessels overlay the tumor area. Segmentation and immediate stagnation of flow occurred during and after coagulation and in 40 percent of the treated eyes small vitreal hemorrhage appeared. The extension of the cautery area was governed by the aim to preserve as much viable retinal tissue as possible, consistent with complete destruction of the tumor involvement. A one-mm.-wide encircling collar of ophthalmoscopically normal retina was cauterized as was done with the choroidal-scleral nodule.

Course after diathermy

The edema of the tumor area increases during the first few days postoperatively and blood flow through the overlying vessels is stagnant. Edema of the adjacent retina is frequent, most often inferiorly. After four to five days there are beginning signs of drying up of the treated areas. The edema decreases, the margins of the zone are more definite, and an atrophic rim, with scattered heavy pigment clumps, arises around the tumor. Vitreal opacities overlying the area were present in all cases.

Retinal lines of tension, as are often seen in human eyes, were absent. The central tumor area becomes a deeper grayish white, and may contract to two thirds of its original extent. In many eyes, in which the neoplasms seemed at first to be well coagulated, it was possible to see a fine roll or rim of pinkish white renewed activity appearing within the white coagulated zone and extending to its margin, or arising at the margin itself. Most of those noted appeared between the superior aspect of the intraocular tumor bed and the optic nerve head. With renewed activity there was an accompanying extensive retinal detachment, increase in vitreal haze, and decreased visible detail.

Only one eye thus treated was negative at time of enucleation. The total time of observation after treatment was greater than two weeks in 20 eyes, and less in two (three and 12 days). Typical scleral cautery changes were seen below the nerve head with either original viable or regrowth of new tumor overlying in many eyes in which sections passed through the original cautery areas. In others, adjoining atrophic choroid and glial retinal changes produced by diathermy were devoid of neoplasm or its debris. The sites of the choroidal-scleral nodules displayed much cellular debris frequently, with heavy calcification and usually with surrounding viable tissue. These areas of viable tumor tissue were mingled with the naturally occurring necrosis, as is seen in all transplants.

Comment

The criteria used of considering that the ophthalmoscopically visible damage to the retina and sclera overlying this tumor was sufficient evidence of lethal damage to Vx2 cells were not accurate. Multiple applications of a small electrode were apparently not sufficient to include each and every neoplastic cell within a coagulating sphere of heat diffusion. The retinal edema was apparently induced by a lower temperature than that required for destruction of the tumor cells, and many gaps were thus left within the body of the tumor and the encircling one mm. border of diathermy treatment.

A diathermy attack on this nonpigmented tumor which does not have clearly outlined borders is limited by the uncertain knowledge of its extent, even under constant ophthalmoscopic surveillance, and by the absolute

necessity of overlapping the effective spheres of coagulating heat throughout the entire neoplasm and its possible microscopic extensions.

Cautery plus chemothermy

The use of chemotherapeutic adjuncts in treatment of retinoblastoma has been presented recently.[12] The cautery treatment of nine eyes with Vx2 carcinoma was supplemented by intra-arterial common carotid injections of HN2 in five, and combined TEM, HN2, and Colchicine in four animals (Fig. 11).

Effects of anticarcinogens on the normal rabbit fundus and brain

The use of HN2 and TEM is palliative and supplementary.[13] French[14] has pointed out the hazards of intracarotid HN2 injection. Its many side effects are known.[13] The tolerance of the rabbit fundus and brain to various doses of HN2, TEM, and Colchicine singly and in combination was investigated.

Method

The common carotid was exposed at the level of the larynx and No. 60 polyethylene tubing inserted and tied into place. A three inch length for future use was left protruding and after the first injection was heat sealed and placed subcutaneously. Sealed tubes have remained patent in unheparinized rabbits for 10 days.

The black blood usually found filling the lumen can be washed out and repeated injections made. It was not possible to perform more than four injections on any one animal because of unavoidable throat infection. Ophthalmoscopic examinations were done following the treatments and microscopic examinations of the eye and brain (in 10) were made at time of death. Table 1 shows that in the rabbit the fundus can tolerate separately one mg. of HN2 and TEM/six lb. weight at daily injections four times and three times, respectively, given every other day.

TEM and HN2, one mg. given combined at the same sitting, cause moderate edema, segmentation, and retinal petechial hemorrhages. Doses higher than this cause severe retinal damage.

Figure 12 shows the marked effect of 2.0 mg. of TEM and HN2, intra-arterially in a six lb. rabbit. Marked sluggishness of the retinal circulation appeared within several hours and after 20 hours there was easily

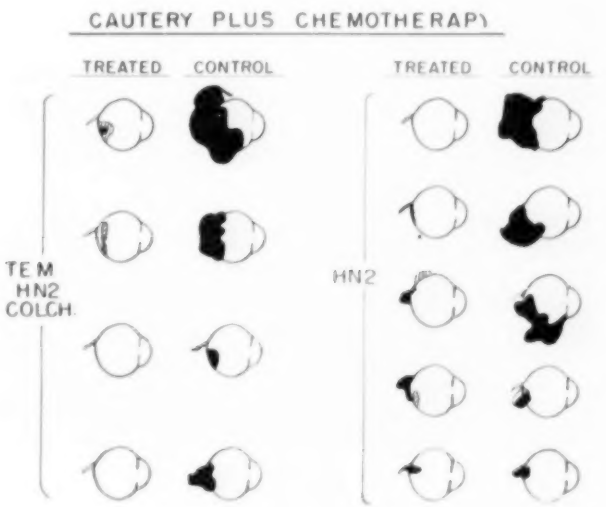

Fig. 11 (Vogel). Cross section of eyes treated with cautery and chemotherapy. Markings are the same as in Figure 9.

TABLE 1

TOLERANCE OF RABBIT FUNDUS

Drug	Dose (mg. per 6 lb.)	Interval (days)	Segmentation	Complications		
				Hemorrhage	Edema	Brain
HN2	¼ ×2	1	–	–	–	–
	½ ×4	1	–	–	–	–
	1 ×3	1	–	–	–	–
	1 ×4	1	–	–	–	–
TEM	1 ×1		–	–	–	–
	1 ×3	1, 2	–	–	+	–
	1 ×3	1, 4	–	–	–	–
HN2+TEM	0.5 @ 1		–	–	–	–
	1 @ ×1		+	+	++	–
	2 @ ×1		+++	++	++	–
	4 @ ×1		++	++	++	
HN2+TEM	0.5 @ +0.25 ×1		–	±	–	
+Colchicine	0.5 @ +0.25 ×3	2, 2	–	–	–	
	1 @ ×1		–	–	–	
	1 @ ×1		++	++	++	
	1 @ +0.5 ×4		–	++	++	

seen segmentation of the vessels of the posterior wing, beginning edema, and petechial hemorrhages. After 72 hours the vessel outlines were completely obscured by hemorrhages, petechial and mulberry, and edema which included the posterior wing and the lower posterior half of the fundus. The damage was confined to the posterior fundus in all those animals examined. A sharp line of demarcation which usually started at the nerve head marked the anterior border of the edematous area.

Adnexal changes were frequent. Decreased lid reflex appeared in all those animals receiving a total of 1.5 mg. + of HN2 intra-arterially. Marked unilateral increase in nasal discharge, lid paralysis, marked mandibular tissue swelling extending down onto

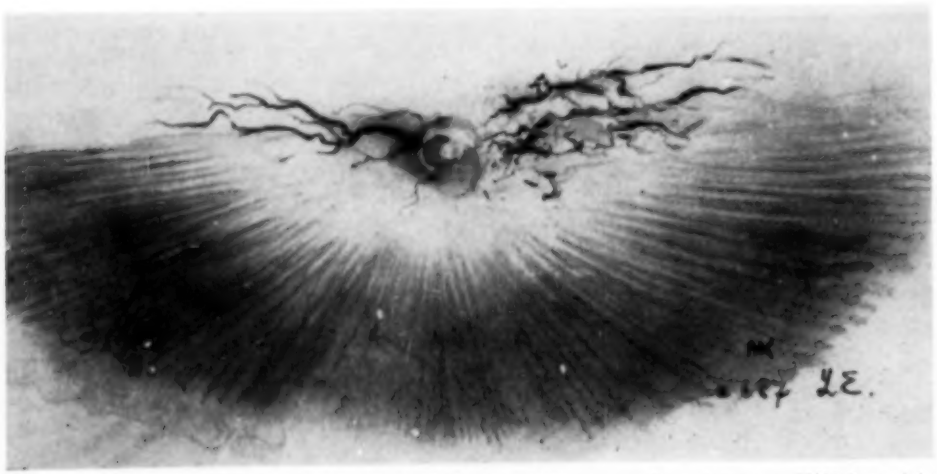

Fig. 12 (Vogel). Right eye, 24 hours after injection of 2.0 rg. TEM and 2.0 mg. HN2 into right carotid artery. Posterior wing of retinal vessels is disorganizing, with petechial and mulberry hemorrhages and edema of the posterior half of the fundus.

the throat was present in all those receiving 3.0 mg. HN2 or 1.0 each of HN2, TEM, and Colchicine. Ataxia and head tilt were seen in several receiving combined TEM and HN2. Unilateral marked hemifacial atrophy was noted in several cases which survived the marked mandibular swelling.

Treatment

All animals were given ipsilateral intra-arterial injections within 24 hours of cautery therapy. Total dosage of HN2 was 2.0 mg. in four animals and three mg. total in one.

Complications were massive mandibular swelling in two, epilation of lid in one, deep corneal ulcer in one. No histologic changes directly attributable to the HN2 could be seen except the severe keratitis and hypopyon found in one case. A total of 1.0, 1.0, and 0.5 mg. respectively of HN2, TEM, and Colchicine were given in combination to four animals at the same sitting intra-arterially, within 24 hours of cautery treatment. In this group one eye was malignant and three were nonmalignant. In these eyes the debris of Vx2 cells could be seen in two, and in one an intravitreal mass of dying poorly staining cells were identified. One eye showing marked damage to the neoplasm also revealed a central viable core over the nerve head.

Comment

These doses of HN2 were within the amounts tolerated by the normal rabbit fundus (table 1). Those animals receiving a combination of drugs were exposed to doses slightly above that tolerated by the normal rabbit fundus. Adnexal complications occurred in six of 11 tested. Brain sections through the cerebellum, mid, and forebrain of 10 rabbits receiving doses ranging from amounts easily tolerated by the fundus to quantities causing severe damage did not show any signs of vascular or stromal injury.

This lack of central nervous system involvement is probably not due to tolerance of the rabbit brain but to shunting of the agents to the facial and submaxillary areas via the relatively large external carotid and maxillary arteries. This group indicates that it may be possible with judicious adjunctive use of anticarcinogenic agents to destroy those neoplastic cells escaping cautery therapy. The general pathologic picture of these treated eyes was not noticeably more severe than that found in the group treated with cautery only.

Chemotherapy only

The only treatment of 11 eyes was intra-arterial injection of anticarcinogen. In eight eyes HN2 in doses ranging from 1.0 mg. to 6.0 mg. was used. In two eyes HN2, TEM, and Colchicine were combined (1.0, 1.0, 0.5 mg., respectively). Table 2 shows the individual dosages and results.

In this group one eye was nonmalignant. Complete destruction of the retina in this eye was in progress at the time of enucleation with a small area showing minimal damage

TABLE 2

Intra-arterial injection of anticarcinogen

Drug	Dose (mg.)	Interval (days)	Neoplasm	Complication
HN2	1		+	—
	2		+	—
	2		+	—
	2		+	—
	1×4	1	+	Ulcer, hypopyon, swelling of throat
	1×4	1	+	Perforating corneal ulcer
	1×4	1	+	Salivation, nasal discharge, lid reflex (−)
	1×4	1	+	Maxillary swelling, lid reflex (−)
	2×3	2, 2, 5	−	Retina completely atrophic
TEM, HN2, Col.	1, 1, 0.5		+	—
	1, 1, 0.5		+	Hemifacial atrophy

TABLE 3

VISION OF TREATED AND CONTROL EYES FROM ALL GROUPS

Therapy	Treated		Control		
	Blind	Light	Blind	Light	
Stallard disc (Large)	2 0	6 0	7 7	1 1	Neoplasm +
Stallard disc (Small)	1 1	6 2	6 6	1 1	Neoplasm +
Stallard disc (combined)	2 1	0	2 2	0	Neoplasm +
Stallard disc + cautery	1 1	2 1	2 2	1 1	Neoplasm +
Cautery	17 16	5 5	18 18	4 4	Neoplasm +
Cautery + chemotherapy HN2	4 3	1 1	5 5		Neoplasm +
Cautery + HN2 + TEM	4 1	0	4 4	0	Neoplasm +
Chemotherapy	9 9	2 2	9 9	2 2	Neoplasm +
Total:	41	22	53	9	
Malignant	32	11	53	9	
Nonmalignant	8	11			

with loss of rod and cone area only. The rest of the eye showed marked retinal glial changes. Adnexa and ocular complications occurred in six eyes.

VISION AT TIME OF ENUCLEATION OF TREATED EYES VS. CONTROLS

Table 3 shows the resulting vision of treated and control eyes from all groups. The criteria of evaluation of vision were on the bases of rough ophthalmoscopic and histologic examinations as follows:

BLIND

Retinal detachment greater than one-half, obscuration of detail by haze of any media, histologic evidence of retinal destruction of rod and cone layer of two thirds of the retina, involvement of the nerve head by neoplasm, beginning glial changes in two thirds of the retina.

LIGHT

(+) Blink reflex: evidence of histologic and ophthalmoscopic integrity of one half of retina.

COMMENT

All the control eyes (contralateral) were malignant in the treated group. Of these, nine, according to the arbitrary grading used, had light perception. Of the treated eyes, 19 were nonmalignant. Eleven had light perception and eight were blind. Forty-three treated eyes from all groups were malignant and of these 11 had light perception.

It is probable that had those eyes with both light perception and malignancy survived, blindness would have developed. Eleven eyes only of all groups (124) were both nonmalignant and with light perception. All of these were from the Stallard disc group.

SUMMARY

The characteristics of growth of a rabbit neoplasm (Vx2 carcinoma) transplanted into the rabbit fundus are described. This lesion is suggested as a possible experimental means

of evaluation of therapeutic weapons against intraocular neoplasms, and experiences with the Stallard disc, cautery, and chemotherapy are described.

Conclusions

1. Double foci of neoplastic cells arose in 80 percent of the Vx2 host eyes. The locations are usually the same and predictable.

2. The intensity of the host response varies in each animal. The contralateral control eyes in seven out of 69 treated animals were negative and these are not included in the discussion.

3. The host response consists of small round-cell infiltration, neovascularization of stroma, scarring, and subretinal serous outpouring. These are not necessarily interdependent, but do follow each other bilaterally in untreated animals.

4. The Vx2 tumor is not supported by the vitreous alone.

5. The most practical and easy medium of transplantation is saline suspension.

6. The most practical approach for ocular transplantation is transvitreal into a subretinal pocket.

7. The normal rabbit eye does not evoke a response to nonviable Vx2 material within four days of transplantation.

8. There is presumptive evidence of an increase in capillary permeability and weakening of capillary walls in the areas immediately adjacent to the tumor.

9. (a) A wide collar margin of safety is required in application of contact radiation over this unpigmented neoplasm. (b) This neoplasm can grow and spread adjacent to a Stallard disc in situ.

10. (a) Cautery with a small (0.5. mm.) electrode requires the overlapping of effective lethal zones of heat diffusion around the tumor borders and through the body of the tumor. (b) This was not accomplished in 21 of 22 cauterized eyes.

11. Chemotherapy in conjunction with cautery may be a sufficient adjunct to eradicate those remaining cells not included in the diathermy barrage.

12. (a) The rabbit fundus can tolerate up to a total of 4.0 mg. of HN2 intracarotid in divided 1.0 mg. doses; (b) TEM is slightly more toxic to the fundus than HN2; (c) rabbit brain did not show signs of damage with doses up to 4.0 mg. of HN2, and to 2.0 mg. HN2 and TEM combined; (d) the local fundal toxicity of TEM and HN2 is increased if given at the same sitting.

13. Combined chemotherapy in conjunction with cautery is more effective than the use of chemotherapeutic agents singly.

14. Chemotherapy intra-arterially in doses large enough to cause rupture of the globe were not effective in controlling this neoplasm.

15. Those treated eyes displaying both light perception and nonmalignancy were from the Stallard disc group.

1601 Spring Garden Street (30).

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Discussion

Dr. T. E. Sanders (Saint Louis): I was not able to make a previous study of this paper so my remarks must be spontaneous. I believe the author has made a contribution in a rather neglected field, that is, the experimental study of intraocular neoplasms. I believe this technique probably can be applied to problems other than the evaluation of radiation therapy. I am thinking particularly of the mechanism of the glaucoma caused by intraocular tumors. I would like to ask Dr. Vogel if any of these animals developed glaucoma.

I think a word of warning is indicated about drawing a parallel between this lesion and the usual clinical one. This tumor probably even remotely resembles only one tumor seen in human beings, that is, the metastatic carcinoma. I think it would be very difficult to make a comparison with the reaction of radiation of this experimental tumor to the most common intraocular tumors, the melanoma, which is notoriously resistant to radiation. Probably the cautery technique would be more applicable.

I think one other point can be made; that is, the extension of the experimental tumor along the needle pathway suggests that indiscriminate biopsies of intraocular tumors should be avoided.

LENS CHANGES FOLLOWING X-IRRADIATION*

Of single and multiple quadrants

R. D. Richards, M.D., E. F. Riley, Ph.D., and P. J. Leinfelder, M.D.

Iowa City, Iowa

It had been observed that X-irradiation of one quadrant or two opposite quadrants of a rabbit lens resulted in partial opacities limited to the irradiated areas, even when exposures of 12,000 r were employed.[1,2] These experiments suggested that the protected portion of the lens may somehow have prevented the progression to a complete radiation cataract. Further exploration of this new concept, the sustaining of the lens by the shielded portion until the irradiated portion can effect a type of recovery, is the subject of this report. This idea offers a new approach to an understanding of the etiology of radiation cataracts, as well as possible application to clinical practice.

* From the Department of Ophthalmology (Dr. Richards and Dr. Leinfelder), Radiation Research Laboratory (Dr. Riley), State University of Iowa College of Medicine. This paper was based on work done under Contract AT (11-1)72 Project I for the U. S. Atomic Energy Commission.

Procedures

Single exposures to X-radiation were employed for all experiments. Earlier experiments were done with the following exposure factors: 200 kv., 5.0 ma., 27 cm. distance from target, 1.0 mm. Al filtration and a rate of 150 r per minute. More recent experiments, which included some repetitions of the previous experiments, were done with the following exposure factors: 250 kv., 30 ma., 29 cm. distance, 0.25 mm. Cu plus 1.0 mm. Al external filtration, HVL of 0.99 mm. Cu and a rate of about 500 r per minute. Exposures represent air doses as measured with a Victoreen r-meter.

With one exception (a black-eyed rabbit), albino rabbits of approximately 2.0 kg. were used for these studies. Irradiation of the lens was limited to one quadrant, two opposite, two adjacent, three or four quadrants by a lead shield (fig. 1) sutured to the sclera of the anesthetized rabbit (intravenous Nembutal). These shields, which were 2.0-mm. thick, transmitted only 3.7 percent of the incident radiation. (Liberal application of an ophthalmic ointment before the shield was secured seemed to minimize the dehydration and subsequent clouding of the cornea.)

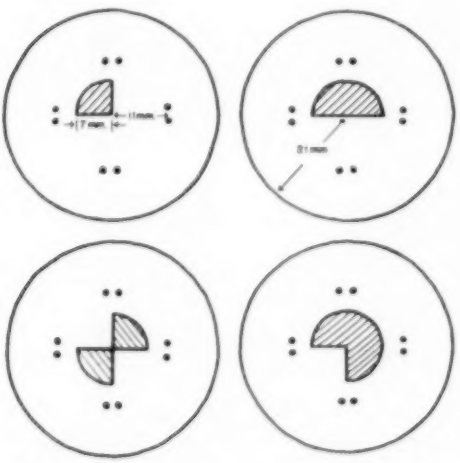

Fig. 1 (Richards, Riley, and Leinfelder). Lead shields used for partial irradiation of rabbit lens.

The rest of the animal was shielded during the exposures by covering with 0.25 inch of sheet lead. After irradiation, rabbits were examined at monthly intervals with an ophthalmoscope.

Table 1 summarizes the number of rabbits used, the dosages employed, and the length of time after irradiation that the lenses have been observed.

Results

After the irradiation of one quadrant, two adjacent or two opposite quadrants, the development of lens abnormalities followed essentially the same pattern. Typically, vacuoles were first observed in the equatorial region of the irradiated portion at about four to eight weeks after irradiation (fig. 2a). The vacuoles were somewhat larger and were observed in the shorter time after the larger doses of X-radiation.

Then, a few weeks after the appearance of the first opacities, vacuolation extended toward the poles (fig. 2b) (posteriorly more than anteriorly) in the subcapsular region of the cortex of the irradiated quadrants. At

TABLE 1

Summary of lens exposures

Quadrants Exposed	Dose, (r)	No. of Lenses†	Months Observed
1	1,500	1	12
	3,000	3	11, 11, 23
	5,000	2	12, 12
	10,000–12,000	3	10, 12, 21
2 Opp.	5,000	1	11
	7,500	1	11
	12,000	4	5, 7, 10, 23
2 Adj.	12,000	4	5, 5, 6, 10
3	11,000–12,000	5	4,* 5, 5, 10, 10
4	1,000	1	5
	1,500	1	6
	3,000	1	3* (blackeyes)
	3,000	1	5*
	5,000	1	3*
	7,000	2	5,* 5*
	12,000	2	4, 4*

* Terminated, complete cataract.
† Includes those previously reported.[3]

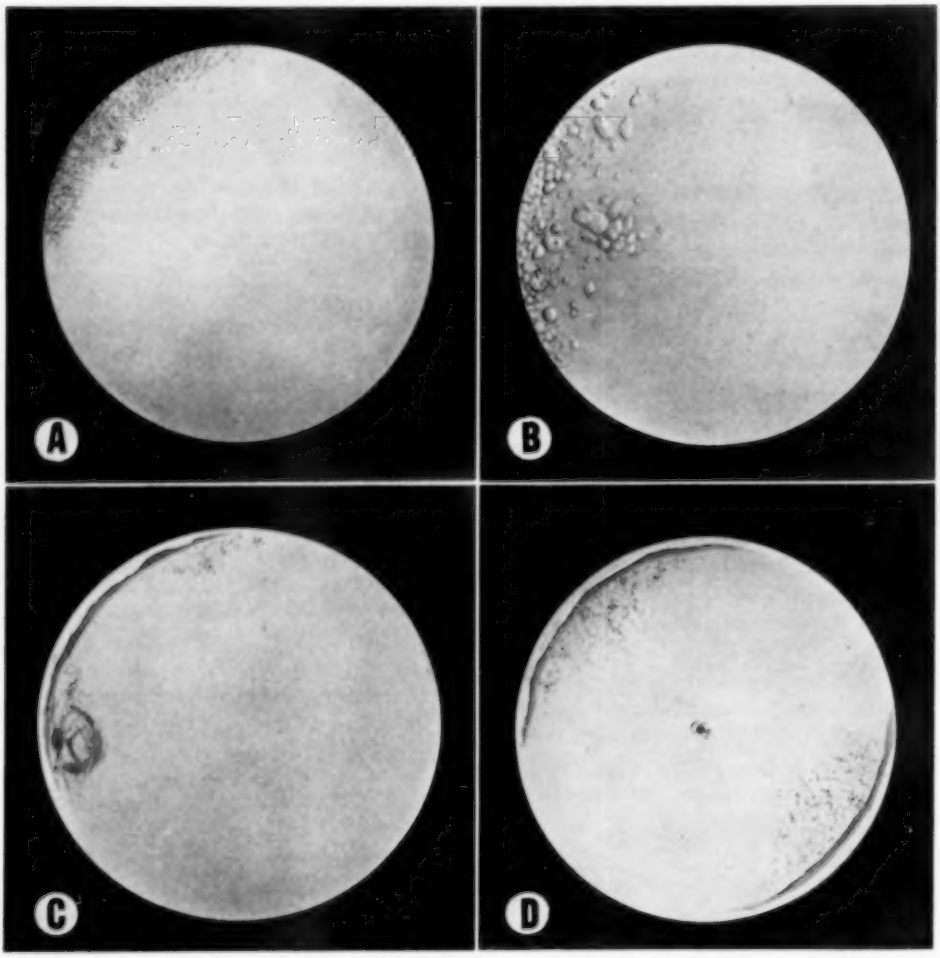

Fig. 2 (Richards, Riley, and Leinfelder). Drawings of partially irradiated rabbit lenses. (A) Six weeks after 5,000 r to one quadrant. (B) Four months after 11,000 r to one quadrant. (C) One year after 3,000 r to one quadrant. (D) One year after 12,000 r to two quadrants.

approximately six months after exposure to 3,000 r or more, an arcuate opacity developed in the equatorial region where vacuoles had first been noted (fig. 3b). Coincident with the appearance of the arcuate opacity, the formation of new vacuoles seemed to stop, and those already present in the irradiated quadrants appeared to be accumulating at the poles.

Six months later, one year postirradiation, the arcuate opacity appeared to be deeper in the cortex and somewhat less apparent. Also, at this time, fewer vacuoles could be seen, and most of these were fine, punctate opacities (figs. 2c and 2d). The general impression was that there had been a regression of the cataract. The shielded portions of the lens remained essentially clear. There was little difference, after 12 months, in the degree of cataract produced by exposures of 3,000 r and 12,000 r.

One year after the irradiation of one

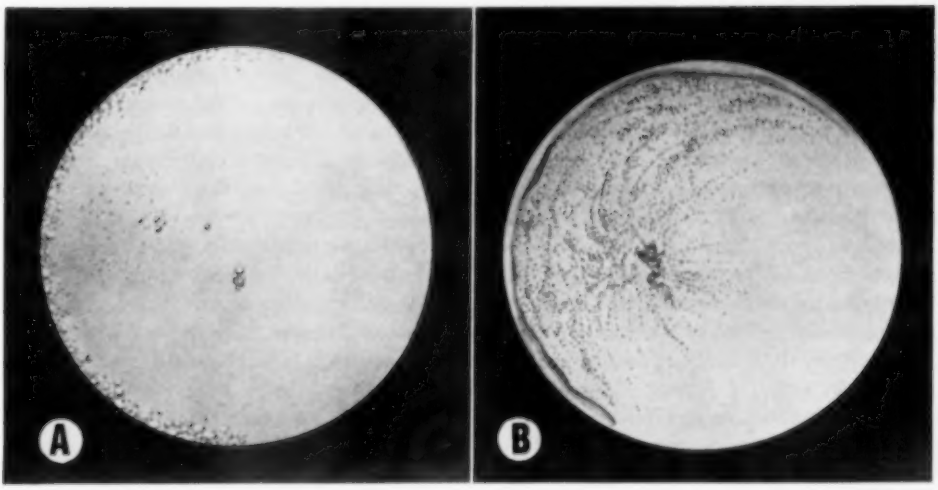

Fig. 3 (Richards, Riley, and Leinfelder). Drawings of partially irradiated rabbit lenses. (A) Two months after 12,000 r to one half. (B) Six months after 12,000 r to one half.

quadrant or two opposite quadrants, several animals were killed and the lenses were removed for histologic study. Before fixation in Carnoy's fluid, the arcuate opacities could be seen at the equatorial region, one to two mm. inside the capsule, and apparently overlaid with normal cortical substance (fig. 4a).

Fairly thick sections of the lens were cut with a freezing microtome, stained with hematoxylin, and mounted in glycerine jelly. One lens with a peculiarly shaped arcuate opacity was sectioned as described.

Microscopic examination of the sections revealed a crescent-shaped area which corresponded in general outline and location in the cortex with previous gross and ophthalmologic observations of the arcuate opacity (fig. 4b). This crescent-shaped area appeared to be filled with a clear, homogeneous, lightly staining substance. Except for an atypical arrangement of the fibers next to the arcuate opacity, the appearance of the fibers between the arcuate opacity and the capsule was nor-

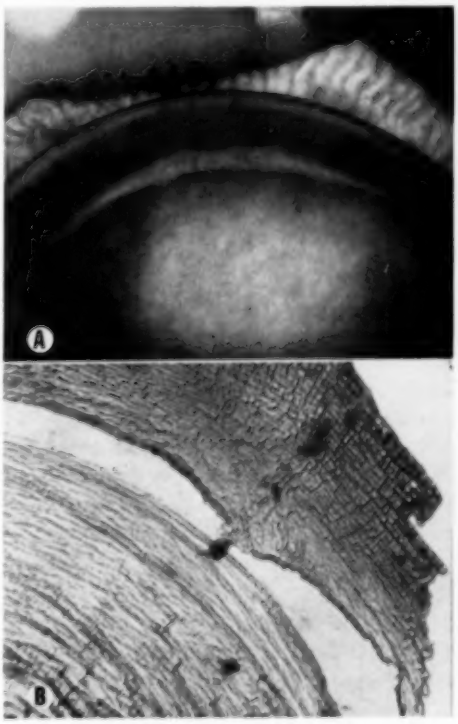

Fig. 4 (Richards, Riley, and Leinfelder) (A) Photograph of arcuate opacity in rabbit lens 11 months after an exposure of 3,000 r to one quadrant. (B) Photograph of arcuate opacity in section of rabbit lens 20 months after an exposure of 11,000 r to one quadrant. Note smoothly curving inner surface and disorder of fibers first covering irregular outer surface.

mal. McDonald, et al., using beta radiation, observed a similar "crescent-shaped homogeneous zone of pink staining material . . . located between the lens nucleus and normal peripheral cortex" which they thought represented "liquefied cortex."[3]

The irradiation of three quadrants produced variable results. One lens developed a complete opacity in four months after exposure. Four of the lenses seem to be following the pattern typical of a one- or two-quadrant injury. Two of the four have been observed for 10 months, twice as long as was usually required for the development of a complete cataract when the entire lens was exposed to 3,000 r or more.

Irradiation of four quadrants of the lens with 3,000 to 12,000 r produced extensive vacuolation in the equatorial region in four to eight weeks after exposure. (A shield, similar to those illustrated in Figure 1, but with four quadrants cut out, was used to give conditions of exposure comparable to the partial lens irradiations.) The equatorial band of vacuoles then widened, with most of the vacuoles seeming to migrate posteriorly. Then, three to five months after irradiation, the lens developed a diffuse cloudiness obscuring detail and progressed rapidly to complete opacification.

Comments

The first changes in a lens exposed to X-radiation are generally thought to be (1) an injury of the epithelial cells located near the lens bow in the zone of greatest mitotic activity, (2) the subsequent failure of these injured epithelial cells to form normal fibers, and (3) the migration of the aberrant fibers toward the poles of the lens.

Clinically, the first changes observed in a rabbit lens are (1) the formation of vacuoles in the equatorial region of the lens and (2) the spreading of the vacuoles from the equator toward the poles of the lens. Considerable evidence has been accumulated which supports this general picture.[4-12]

In our studies the irradiated rabbit lenses all followed this general pattern of early change. When only a portion of the lens was irradiated, the changes occurred only in the irradiated portions. With comparable doses, the sequence of events and the time required for these first changes were the same whether one, two, three, or four quadrants were exposed.

Although all the rabbit lenses followed the same pattern of *early* changes, a divergence in the progression of the opacities became evident at three to five months. At this time, the opacities in lenses exposed in all portions to 3,000 r or more progressed rapidly to completion. In contrast, the opacities of lenses irradiated in only a portion of the lens stopped progressing and eventually appeared to regress. Exposure of the entire lens to 1,000 r also resulted in an incomplete opacification. Apparently, after low doses to an entire lens, or more massive doses to only a portion of the lens, the lens is again able to produce normal fibers at some time after irradiation. With larger doses to the entire lens, the formation of pathologic fibers apparently continues and eventually even the degeneration of fibers formed prior to irradiation may occur. In the later stages of the progression to complete opacification, the lens becomes hydrated and others have observed an increased uptake of radiosodium and radioiodine.[11,13]

At this point in our study, we do not know how the shielding of a portion of the lens can prevent the progression to complete opacification, even after exposures to 12,000 r. Several explanations seem to be possible. Three possibilities, in brief, are: (1) replacement of the injured epithelial cells by proliferation of the cells in the central or in the shielded portion of the epithelium, (2) a sustaining of the lens by the unimpaired metabolism in the shielded portion, and (3) a dilution or nullification of catabolic products from the injured portion by the protected portion. We also recognize that still another explanation may eventually be shown to be the correct one.

The first of the possible explanations, the replacement of injured epithelial cells by proliferation of undamaged epithelial cells, must certainly be one factor which enables the partially irradiated lens to effect a type of recovery. That the epithelial cells are damaged in the irradiated portion is indicated by the formation of aberrant fibers subsequent to the irradiation. In fact, the smoothly curving inner surface of the arcuate opacity suggests a sudden and complete cessation of normal fiber production in the irradiated portion.

Yet, despite this extensive injury, the irradiated portion of the lens regains the ability to produce normal fibers; this is evident in histologic sections where normal fibers can be seen covering the arcuate opacity. The replacement of injured epithelial cells could have occurred through a proliferation of cells from the shielded, peripheral zone of the epithelium. However, this seems unlikely since the time required to replace the injured cells in three quadrants was about the same as was required for the replacement of cells in one quadrant.

It seems more plausible that the replacement of the injured peripheral cells was accomplished in part, at least, by a proliferation of cells in the central zone of the epithelium. This zone has been shown to be relatively inactive mitotically[5, 14, 18] and relatively insensitive to ionizing radiation.[1, 6, 7, 15] So, even though a portion of the central epithelium was exposed to 12,000 r, it may have provided at least part of the reserve supply of epithelial cells. This cannot be the entire answer, however, since the central zone of the epithelium cannot repair the injury produced when the *entire* lens is exposed to much less radiation, 3,000 r or more.

The second possible explanation is that the metabolism of the shielded portion sustains the entire lens during the critical period after X-irradiation; this critical period is apparently at three to five months for the rabbit lens. The lens may be like other body organs, for example the liver or kidney, which function at capacity only when under stress. If so, the shielded portion may be able to increase its rate of metabolism sufficiently to maintain the entire lens until a recovery has been effected. That there is a decreased rate of metabolism in the later stages of cataract development is indicated by the hydration of the lens and an increased uptake of radiosodium.[16, 17] Perhaps, when a cataract goes to completion, this decreased rate of metabolism may also be responsible for the failure of the epithelium in its continued efforts to regenerate and resume normal fiber production.

Another possible explanation is that the shielded portion in some way counteracts substances accumulated or released in the injured part. Histologic examination of lenses which had developed complete cataracts showed autolysis of fibers formed prior to irradiation. This suggests a secondary change in the deeper portion of the lens as a result of radiation injury. If this is the case, the shielded portion may then act in some way to prevent the action of these deleterious agents.

None of these possibilities seems adequate in itself. We feel, however, that each possibility is plausible and that all three may be involved in the complete explanation of the development of a radiation cataract.

Summary

Shielding of at least one-half of a rabbit lens results in a partial opacity restricted to the X-irradiated portion, even with doses of 12,000 r. Histologic examination of such lenses at one year after irradiation shows that normal lens fiber production has been resumed in the irradiated portion. The shielding of only one fourth of the lens does not always prevent the formation of a complete radiation cataract.

University Hospitals.

Acknowledgment

We wish to thank Dr. T. C. Evans for his help in preparing sections of the lens and Mr. Howard Webster for the drawings.

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Discussion

Dr. Ludwig von Sallmann (Bethesda, Maryland): First, I would like to say that it is a privilege to discuss a paper dealing with a problem of radiobiological impact. Any contribution to this topic comes in good time, as hazards of exposure to ionizing radiations are increasing at a considerable rate.

At present this phenomenon can be studied best on a morphologic level, an approach followed by Richards and co-workers, but at variance with earlier work on recovery potentialities of the irradiated lens by others; the investigators attacked the problem in a new and intriguing manner. As you have heard, they exposed some sectors of the lens, while shielding others, and, unexpectedly, observed that doses about 10 times greater than the average cataractogenic dose for the rabbit failed to produce extensive and progressive lenticular opacities, provided 50 percent or more or the lens body was shielded.

No satisfactory explanation of these results could be given. This is not surprising if one recalls the unsolved enigma of the protection phenomenon in total body irradiation by shielding the spleen or other parts of the body. In the case of the lens, the main stumbling block for an adequate interpretation lies in the very nature of radiation-induced lesions of the lens epithelium.

Such injuries of this cell layer caused by a dose of only 1,000 r X rays, are not confined to the cell population which was exposed, but are propagated from generation to generation for many months, with a slow decrease of the number of pathologic mitoses and degenerate cells. Migration of epithelial cells into superficial cortical layers also continues for a long period. As you know, these cells either disintegrate and disappear, or give rise to freak lens fibers which are the substrate of early lenticular opacities.

It is difficult to understand, therefore, how it is possible that the newly formed lens fibers can possess normal physical properties when the radiation damage to the germinative zone must have been excessive. The answer to the question might come from a systematic study of the various changes on a cytologic level.

One point should be made in regard to final evaluation of radiation effects of doses as high as

20,000 r; that is, that such an appraisal requires an extension of the observation time; it can be said now, however, that the reported results may well have broader radiobiological significance than can be visualized at the present moment.

With respect to regression of lens opacities, I would like to stress the discrepancy between clinical, that is, slitlamp and microscopic observations. In various types of experimental cataract, a homogeneous layer of possibly liquefied lens fibers in the supranuclear zone disrupts the normal histologic architecture of the lens. The following slide illustrates such a change—which is similar to the one described by Richards after irradiation—in lenses of rats which had been kept on a 25-percent galactose diet for one week only. The diet-induced subcapsular vacuoles had vanished, and diffuse moderate opacities had almost disappeared when the animals were studied clinically about two months later, but histologic examination showed the severe structural damage in an almost clear lens. It can be said, then, that the terms "recovery" and "regression" should be defined as to the method of examination which was used.

THE EFFECT OF MODERATE DOSES OF X-RAY IRRADIATION ON OCULAR TISSUE*

BERNARD BECKER, M.D., MARGUERITE A. CONSTANT, PH.D., PAUL A. CIBIS, M.D., AND MICHEL TER-POGOSSIAN, M.D.
(With the technical assistance of MISS ROSALIE SMITH)
Saint Louis, Missouri

Radiation damage to the mitotic cells of the germinative zone of lens epithelium has been demonstrated by von Sallmann.[1] Cysteine was noted to alter the effects of irradiation on mitotic cells of lens epithelium and to offer protection against the development of cataracts.[2] These findings suggested the usefulness of measurements of mitotic activity for the study of irradiation effects and their prevention.

The role of ciliary body damage in the development of lens alterations was suggested from the shielding studies of Puntenney and Shoch.[3] Increased permeability of the blood-aqueous barrier to fluorescein was observed following irradiation with 250 r.[4] Kinsey and co-workers[5] indicated that plasma ultrafiltrate was a poor medium for maintaining mitotic activity of cultured lenses in vitro. Furthermore, Brown[6] observed the appearance of a cellular reaction in the anterior chamber following radiation. The question, therefore, arose as to the effects on mitosis in vivo of the entrance of plasma products and cells into the anterior chamber. It is the purpose of this report to demonstrate a remarkable similarity in time course and severity of the exudative process, the alteration of secretion of aqueous humor, and the inhibition of mitosis following various doses of irradiation to the eye.

METHODS

Adult, male, albino rabbits (Haskins Rabbitry, Saint Louis), weighing approximately two to three kg., received various doses of irradiation (100 to 1,300 r). A deep therapy X-ray unit operated at 200 keV peak, 20 ma., 0.25 mm. Cu plus 1.0 mm. Al filtration, HVL. 0.65 mm. Cu, 188 r/min. at 30 cm. distance was used. Irradiation was delivered in single doses to the unanesthetized animal. The beam was collimated to a diameter of 2.0 cm. through a copper collimator approximately 1.0-mm. thick and 2.0 cm. in diameter which was centered over the superior aspect of the globe and approximately 2.0 mm. away from the skin. Scatter was estimated to be less than five percent to the contralateral eye and so opposite eyes served as controls.

At time of death, the anterior chamber

* From the Department of Ophthalmology and the Oscar Johnson Institute, Washington University School of Medicine. This research was supported in part by the USAF under Contract No. AF 18 (600)-1269; monitored by the USAF School of Aviation Medicine, Randolph Field, Texas.

fluid was withdrawn and two aliquots of 0.03 cc. were allowed to dry, then stained with Romanowsky's stain. The number of cells in the aliquot were counted and appropriately adjusted to cells per anterior chamber (0.2 cc. taken as average total anterior chamber volume).

Tonography was performed on the unanesthetized animal (except for local tetracaine), using an electronic tonometer connected to a Leeds and Northrup recorder. Tonographic tracings were obtained on the irradiated eye first, contralateral eye second, in almost all instances.

Flat preparations of lens epithelium were made according to the method of Howard as modified by von Sallmann.[7] Slitlamp examinations were carried out with a Zeiss-Opton instrument.

Results

A. The exudative reactions

1. *Nature and occurrence*

Following irradiation (100 to 1,300 r) to the rabbit eye, a cellular exudate was observed to appear in the anterior chamber. This exudation began typically as a pouring of cells into the anterior chamber from the superior margin of the pupil, covering the lens surface in curtain fashion. During the early period (for approximately 12 hours) a large proportion of the cells were observed to be freely moving with the convection current of the aqueous. Subsequently they became adherent to the lens surface. At approximately 24 hours the major portion of the exudate was concentrated at the upper one half of the lens surface. It frequently formed an inverted triangle configuration with apex at the center of the anterior capsule of the lens. A photograph of the clinical appearance of cells on the anterior surface of the lens 24 hours after a radiation dose of 500 r is presented in Figure 1. The histology of the exudate is demonstrated in a photomicrograph of a flat preparation of lens epithelium (fig. 2). This was made by carefully stripping the epithelium from an unfixed lens onto a cover slip. Numerous white blood cells and macrophages engulfing debris were found adherent to the capsule pre- and postequatorially for the whole circumference of the epithelium.

Examination of stained smears of aqueous, withdrawn after doses of 100 to 500 r, revealed that the exudate consisted principally of white blood cells. At doses above 500 r an increasing proportion of red blood cells was found. The inflammatory nature of the exudation could also be demonstrated by the selective accumulation of Evans blue at the site of intradermal injection of aqueous removed 24 hours after irradiation. Th is the technique described by Menkin for demonstrating leukotaxine.[8]

In a given series of animals with various doses of irradiation, the correlation of sever-

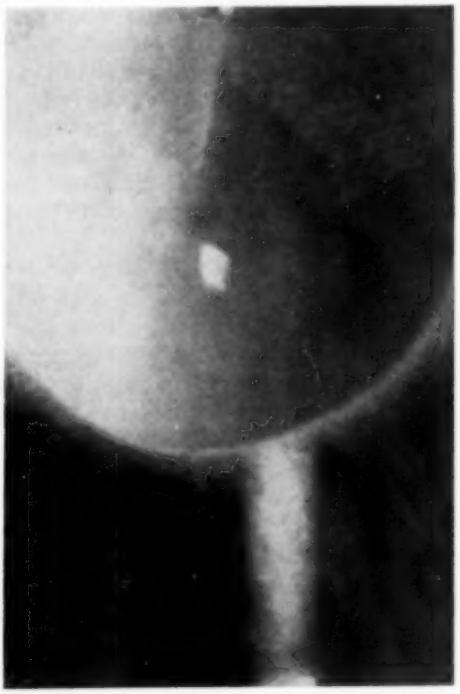

Fig. 1 (Becker, Constant, Cibis, and Ter-Pogossian). Photograph of the clinical slitlamp appearance of the inflammatory response on the anterior surface of the lens (24 hours after 500 r; magnification ×50).

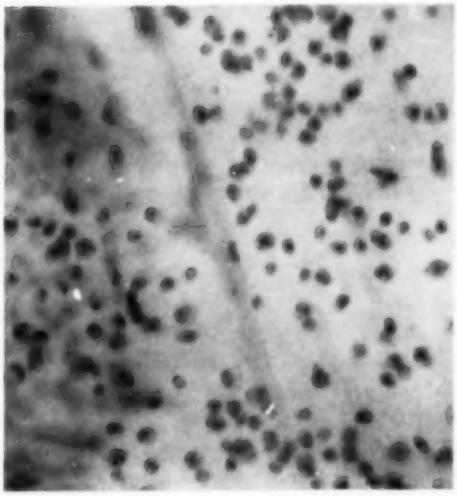

Fig. 2 (Becker, Constant, Cibis, and Ter-Pogossian). Photomicrograph of histologic appearance of exudate on capsule of lens showing its inflammatory character. Cells out of focus on the left are epithelial cells of the lens (24 hours after 500 r; magnification ×200).

ity of exudation with intensity of dose was very good. However, as indicated by the data in Table 1, when several of these experiments were conducted over a period of many months, some individual animal variations in the severity of the inflammatory response to a given dose were observed. In general, a minimal number of cells was observed after less than 200 r (0 to 100 cells) and a nearly maximal exudation (10^4 cells) usually followed 500 r. Larger doses caused a more dense exudation which was occasionally enmeshed in a fibrinous clot. The inflammatory response was evident in two to four hours after the larger doses (500 r or more), but was delayed in onset following doses of 300 r or less. Even after 700 to 1,300 r, the quantity of the cellular exudate was reduced to the order of hundreds by the third and fourth day. However, residual exudation was still adherent to the lens surface of the majority of animals at 10 days. In a long-term study after 1,800 r, anterior subcapsular cataractous changes frequently assumed the configuration of this residual exudate.

2. *Effects of adrenal steroids*

In attempting to modify the inflammatory response, cortisone or prednisolone was administered in daily intramuscular injections of eight to 16 mg. for one to three weeks.

TABLE 1

SEVERITY OF EXUDATIVE PROCESS IN ANTERIOR CHAMBER FOLLOWING IRRADIATION OR PARACENTESIS

Treatment	Time (hr.)	No. of Animals	Distribution of Number of Cells in Anterior Chamber				
			0	10^1	10^2	10^3	10^3+
100–200 r	4–6	9	3	2	4	0	0
	9–11	10	2	3	5	0	0
	28–30	9	0	5	3	1	0
300 r	4–6	7	0	2	1	4	0
	9–11	9	0	1	4	3	1
	28–30	7	0	1	2	4	0
500 r	4–6	5	0	0	0	0	5
	9–11	9	0	0	0	2	7
	28–30	10	0	0	1	4	5
500 r + steroids*	9–11	19	0	5	8	4	2
Paracentesis	28–30	16	0	1	5	4	6
700–1300 r	28–30	8	0	0	0	0	8
	72	22	0	2	14	3	3
	102	6	0	2	2	2	0
	192	6	0	1	4	1	0
	240	6	0	1	5	0	0

* 8–16 mg. cortisone or prednisoline daily for 3 weeks prior to irradiation.

The inflammatory response to a radiation dose of 500 r was markedly reduced in the majority of animals by such pretreatment. Various dose levels and times of injection were similarly effective in prevention of the inflammatory response. Whereas seven of nine animals without pretreatment showed the presence of 10^4 or more cells at 10 hours, only two of 19 animals pretreated with steroids showed a similarly severe exudation.

3. *Effects of paracentesis*

In order to produce an alteration in blood aqueous barrier without radiation damage, a number of rabbits were subjected to paracentesis. Following the withdrawal of 0.10 to 0.15 cc. of aqueous humor, hundreds to thousands of cells were observed in the anterior chamber. However, stained smears of aqueous of these animals showed that the cells were almost exclusively erythrocytes. Furthermore, Evans blue was not attracted to the intradermal site of injection of aqueous, removed 24 hours after paracentesis.

4. *Vitreous exudate*

Slitlamp examination of the posterior surface of the lens and of the vitreous showed the presence of a similar exudative reaction. However, this was delayed in onset and remained for a much longer period. The cells were generally in close proximity to the entire posterior capsule, but in severe cases extended far back into the vitreous. This exudation was seen only occasionally after 500 r, but following 700 r, hundreds of cells appeared after 48 hours. After 1,000 to 1,300 r, thousands of cells were present in 24 hours and were still evident in large numbers at the end of the experimental period (10 days). In the preliminary study, in which a dose of 1,800 r was used, exudation into the vitreous was evident for three weeks, much as observed by Brown[6] after Co^{60} gamma irradiation.

Although it was hoped to use the inflammatory response as a criterion for quantitating various types of irradiation, individual animal variation in cellular exudation, following a given dose of X rays, precluded this application. However, the anterior chamber reaction in conjunction with the appearance of exudate in the vitreous provided an approximation of the dose received.

B. Mitotic activity

Studies of the mitotic activity of lens epithelium at various time intervals after irradiation demonstrated marked reduction in total number of cells in mitosis as compared to the control eye (table 2). Mitotic activity was depressed in the epithelium of most radiated eyes at 10 hours and almost completely inhibited by 30 hours following radiation. The inhibitory effect of higher doses tended to be greater than that following the lower doses.

A comparison of inhibition of mitosis with the inflammatory response in the same eyes revealed that the maximum inhibition of mitosis was evident at a time when the exudative process became adherent to the lens surface. However, mitosis inhibition was almost as great in animals which received 100 to 200 r as it was after 500 r, although the cellular exudation following 100 to 200 r was considerably less than that caused by 500 r (table 1). The lack of dependence of degree of mitosis inhibition on severity of the exudation was more evident in data obtained after pretreatment with steroids or after paracentesis. Although pretreatment with steroids considerably reduced the inflammatory response (table 1), seven of eight animals so treated showed significant inhibition of mitosis (table 2). On the other hand, paracentesis caused an outpouring of many (10^4) cells into the anterior chamber, but the mitotic activity of the lens epithelium of these eyes was not inhibited.

Studies of the correlation of the time of recovery of mitosis and the removal of the exudate from the anterior chamber were made following radiation doses varying from 700 to 1,300 r (tables 1 and 2). The number

TABLE 2

MITOTIC ACTIVITY OF LENS EPITHELIUM FOLLOWING IRRADIATION OR PARACENTESIS

Treatment	Time (hr.)	No. of Animals	Distribution of Number of Cells in Mitosis (irradiated/control)					
			0–0.3	0.3–0.6	0.6–0.8	0.8–1.2	1.2–1.5	1.5+
0	0	15	0	0	0	15	0	0
100–200 r	4–6	9	0	1	1	4	2	1
	9–11	9	0	2	6	1	0	0
	28–30	9	5	4	0	0	0	0
300 r	4–6	6	0	1	2	3	0	0
	9–11	9	0	5	3	1	0	0
	28–30	6	6	0	0	0	0	0
400–600 r	4–6	5	1	1	2	1	0	0
	9–11	13	2	7	4	0	0	0
	28–30	6	5	1	0	0	0	0
500 r + steroids*	9–11	8	4	2	1	1	0	0
Paracentesis	24	8	0	0	0	6	0	2
	48	4	0	1	1	2	0	0
	96	4	0	0	0	4	0	0
700–1300 r	76	6	6	0	0	0	0	0
	102	6	5	0	1	0	0	0
	192	6	1	0	1	2	1	1
	240	6	0	0	0	1	3	2

* 8–16 mg. cortisone or prednisoline daily for 1–3 weeks prior to irradiation.

of animals used at individual dose levels in this range were too few to make it possible to distinguish conclusively between these doses on the basis of inflammatory response or mitosis inhibition. Mitotic activity of the irradiated epthelium was observed to recover shortly after the number of inflammatory cells in the anterior chamber had decreased to a few hundred or less. An increase in number of mitosis to greater than normal levels was uniformly seen at 10 days, confirming the similar observations of von Sallmann, et al.[9]

C. Rate of aqueous secretion

The effect of irradiation upon the secretory process was studied by tonographic tracings. Attempts were made to correlate alterations in aqueous flow with the exudative process and mitotic inhibition. Following 550 to 750 r, most animals showed more than a 50-percent suppression in rate of aqueous flow of the irradiated eye which usually persisted for 48 hours and tended to recover by 72 hours (table 3). Occasional animals had persistent inhibition of flow for up to 96 hours. At higher doses (1,000 to 1,300 r) *all* animals showed a marked suppression of aqueous flow of the irradiated eyes and this persisted up to 192 hours. In six of the 10 animals, flow was decreased more than 75 percent compared to the control eye or compared to the rate of flow in the same eye before irradiation.

Pretreatment with steroids appeared to prevent in part the suppression of aqueous flow. Paracentesis caused a decrease in the rate of aqueous flow in both the treated and control eye. However, the rate of flow in the tapped eye was not significantly different from that of the control eye in spite of the large differences in cellular response.

Discussion

Direct damage to the lens is regarded by most investigators to be a major cause of irradiation cataract.[1] The accessory factors related to the direct or secondary effects of irradiation on the ciliary body remain un-

TABLE 3

THE EFFECTS OF IRRADIATION OR PARACENTESIS ON INTRAOCULAR PRESSURE AND THE RATE OF AQUEOUS FLOW

Treatment	Time (hr.)	No. of Animals	Mean Intraocular Pressure (P_o) (treated/control)	Mean Aqueous Flow* (treated/control)	Distribution of Aqueous Flow (treated/control)			
					0–0.25	0.26–0.50	0.51–0.75	0.76–1.00
550–750 r	0	8	1.00	1.06	0	0	0	8
	24	7	0.81	0.53	2	1	2	2
	48	8	0.85	0.79	0	2	4	2
	72	8	0.86	1.12	1	1	1	5
	96	6	0.85	0.90	0	0	3	3
550–750 r plus steroids†	0	9	1.00	1.02	0	0	1	8
	24	6	0.86	0.75	0	1	2	3
	48	8	0.83	0.80	0	1	1	6
	72	9	0.95	0.95	0	0	2	7
1000–1300 r	0	6	1.00	1.00	0	0	0	6
	72	10	0.55	0.19	6	4	0	0
	144–192	6	0.87	0.78	0	3	1	2
Paracentesis	0	13	0.91	0.86	0	0	3	10
	3	13	1.00	1.23[a]	0	2	2	9
	24	6	1.00	0.96[a]	0	0	3	3

* Flow calculated from $(P_o - 9) \times$ outflow facility.
† 8 mg. prednisoline or 10 mg. ACTH daily for 4 days prior to irradiation
[a] = Flow was decreased 25–40% in both eyes as compared with 0 time.

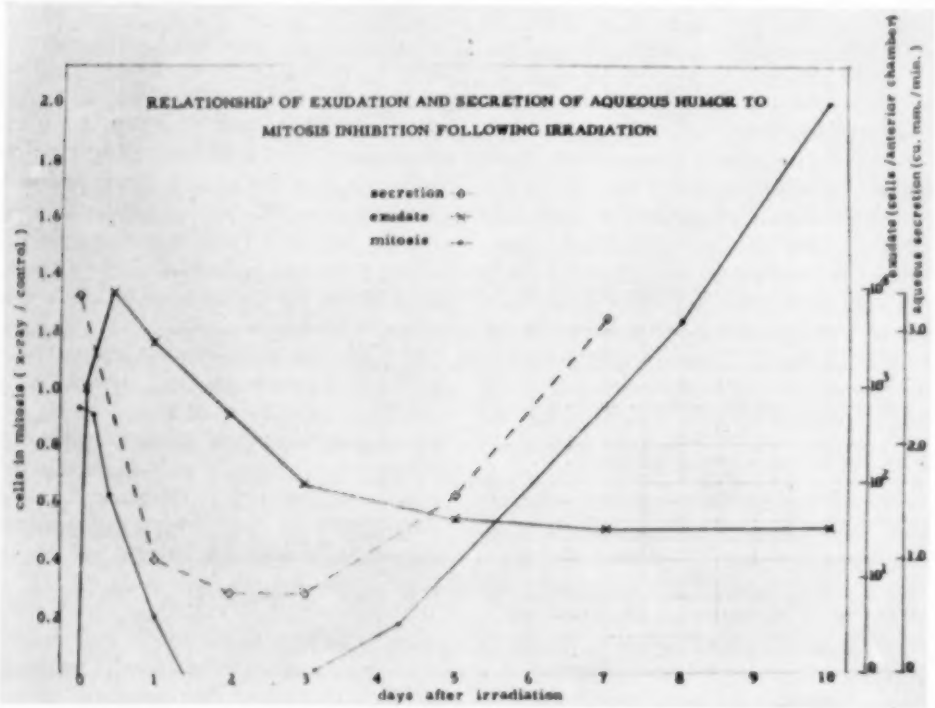
RELATIONSHIP OF EXUDATION AND SECRETION OF AQUEOUS HUMOR TO MITOSIS INHIBITION FOLLOWING IRRADIATION
secretion
exudate
mitosis
cells in mitosis (x-ray / control)
exudate (cells / anterior chamber)
aqueous secretion (cu. mm. / min.)
days after irradiation

Fig. 3 (Becker, Constant, Cibis, and Ter-Pogossian). The relationship between the time-course and severity of the inflammatory response, secretion of aqueous, and inhibition of mitosis following irradiation to the eye (approximately 1,000 r).

proved. The contribution to the cataractous process of suppression of aqueous secretion and the inflammatory reaction needs further elucidation. The over-all correlation found in the present study between changes in mitotic activity, inflammatory response, and suppression of aqueous flow following a cataractous dose of irradiation to the eye would seem to indicate relationships between these processes, but cause and effect are difficult to unravel (see fig. 3).

Support for the idea that the inflammatory response results from the lens damage and is not a cause of mitotic inhibition was obtained from paracentesis studies. This procedure caused a severe exudation into the anterior chamber which consisted almost exclusively of erythrocytes. This type of response was not associated with an inhibition of mitosis or a marked, prolonged suppression of aqueous flow. These data indicated that the failure of plasma constituents in vitro to maintain mitotic activity[5] was probably not a factor in in vivo inhibition of mitosis.

Although the inflammatory response was partially blocked by pretreatment with steroids, the acute effect of irradiation on inhibition of mitosis remained similar to that of nonsteroid-treated animals. It was of interest to observe, however, that the secretion of aqueous humor in these animals tended to be less affected. Long-term experiments are in progress in order to determine whether pretreatment with steroids alters the rate of recovery of mitosis or the development of cataracts. The observations to date tend to support the thesis of a direct effect of irradiation on the lens epithelium with an associated inflammatory response and partial suppression of aqueous secretion.

Summary

Irradiation of the eye with doses of 100 to 1,300 r resulted in an inflammatory exudation into the anterior chamber, a partial suppression of secretion of aqueous humor, and an inhibition of mitotic activity of lens epithelium. There was an excellent correlation in time of occurrence and recovery of these alterations and a tendency for them to be more marked with increasing doses of irradiation.

Although the exudate and to some extent the suppression of secretion were blocked by pretreatment with adrenal steroids, the inhibitory effect of irradiation on the mitotic activity of lens epithelium was not altered by such treatment.

Paracentesis caused exudation into the anterior chamber of similar severity to that observed following large doses of irradiation. However, this exudation was found to consist primarily of erythrocytes and was not associated with an inhibition of mitosis or significant unilateral suppression of aqueous flow.

640 South Kingshighway (10).

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Discussion

Dr. William F. Hughes (Chicago): In their nice study, these investigators have done much to explain possible cause and effect relationships of the several reactions which follow X-ray exposure of the eye. These include an apparent partial breakdown of the blood-aqueous barrier, the formation of an aqueous rich in protein and inflammatory cells, and direct effect of the radiation on the sensitive equatorial cells of the lens epithelium.

Their findings of a similar time relationship between inhibition of mitosis of the lens epithelium, the deposition of many inflammatory cells on the lens, and the suppression of aqueous secretion suggest a possible cause and effect relationship between the three reactions. However, the authors have realized that "all is not gold that glitters," and have shown that the exudate deposited on the lens capsule is not an important cause of inhibition of mitosis, because mitoses were still inhibited in those experiments where exudation was eliminated by steroid therapy.

So far as I know, the authors are the first to demonstrate that the exudation following irradiation contains a leukotaxinlike substance. One is tempted to speculate that leukotaxin may be important in changing the permeability of the lens capsule and perhaps aggravating destruction of cells partially damaged by radiation, or by promoting clouding of the lens cortex.

This recalls the work of Puntenney and Shoch in 1954, in which they showed that exposure of the whole eye to 4,000 r of X rays produced mature cataracts in rabbits, whereas, if either the lens or ciliary body was shielded, the cataracts following exposure to the same dosage only consisted of granular opacities and vacuoles confined to small areas in the subcapsular cortex. They stated that "the presence or absence of normal aqueous may have a profound effect in modifying damage done to the epithelium of the lens and in maintaining the viability of both the old and the newly formed fibers."

Dr. Becker and his associates stated in their manuscript that "the inflammatory response results from lens damage, and is not a cause of mitotic inhibition," partly on the basis that no inhibition of mitosis could be found following paracentesis which produced an aqueous rich in protein and red blood cells. Perhaps this statement is more positive than the authors intended. At least, the following objections to this statement can be offered:

1. The paracentesis fluid is not the same as the aqueous following irradiation because one contained red blood cells and the other contained leukocytes, and the postradiational fluid also contained leukotaxin. However, it is noteworthy that the high protein content of both fluids apparently did not inhibit mitosis, such as Kinsey found in vitro.

2. It seems unlikely that the inhibition of mitosis alone could evoke an inflammatory reaction, although a later breakdown in lens cortex might do so. It seems more likely that the radiation affected the blood-aqueous barrier, allowing the exudation of cells and proteins into the aqueous, rather than that the inflammatory reaction was secondary to the inhibition of mitosis.

The authors stated that pretreatment with steroids appeared to prevent in part the suppression of aqueous flow. In looking over their figures in Table 3, is there really a significant difference between these two groups of eyes, in which it appears that the aqueous flow in irradiated eyes treated with steroids is the same at 48 hours and less at 72 hours than in the radiated eyes which were not treated with steroids?

One last question concerns whether there is any explanation as to how the aqueous flow can be suppressed, as determined by tonography, in view of previous evidence of breakdown of the blood-aqueous barrier to such things as fluorescein, Evans blue, radioactive sodium and potassium as shown by von Sallmann, serum proteins, and inflammatory cells. On the surface, it would appear that a breakdown of the blood-aqueous barrier might increase the rate of aqueous formation.

I want to thank the authors for the privilege of discussing their paper, which helps crystallize some of the etiologic problems of radiational cataract.

Dr. Marguerite A. Constant (Saint Louis) closing: It is true that steroid pretreatment does not alter significantly the inhibition of mitosis. However, the number of animals in our series are far too few, and there is much overlapping of data.

In regard to pretreatment with steroids and aqueous flow, there is primarily the tendency for a more rapid recovery to pre-irradiation rates. Long-term follow-ups are definitely indicated.

In relationship to the rate of aqueous flow versus the more rapid appearance of fluorescein, X rays do increase premeability of the blood-aqueous barrier and provide a faster exchange between plasma constituents and aqueous. However, increased diffusion of substances into and out of the eye does not necessitate increased secretion of aqueous. In fact, increased permeability is usually associated with a decreased secretory rate.

EXPERIMENTAL FUNGUS INFECTIONS OF THE CORNEA*

A PRELIMINARY REPORT

ALBERT P. LEY, M.D.†
Saint Louis, Missouri

There have been several warnings of the dangers of the use of cortisone in ocular infections, namely, in articles by Woods,[1] Hogan, et al.,[2] Offret and Massin,[3] Moser,[4] and Allen.[5] Recently a few case reports of fungus keratitis have appeared in which cortisone was implicated as an accessory. Most suggestive are the cases reported by Mitsui and Hanabusa[6] and one by Thygeson, et al.,[7] in which fungus keratitis supervened de novo during the course of topical cortisone therapy for a variety of other ocular conditions. In the cases reported by Ley and Sanders[8] it could only be inferred that cortisone acted to facilitate existing fungus infections. Moreover, there are no available experimental data on the role of cortisone and antibiotics in fungus infections of the cornea. In contrast, there is a growing body of experimental (and clinical) evidence that cortisone and antibiotics may facilitate systemic fungus infections.

Using mice Seligman[9] demonstrated that almost all the animals died when given cortisone subcutaneously and coincident with intraperitoneal injection of Candida albicans; whereas, animals not given cortisone survived the same number of intraperitoneal organisms. Vogel, et al.,[10] noted that guinea pigs, which have a high resistance to Histoplasma capsulatum, show a marked systemic dissemination of the fungus when cortisone was administered just prior to or following inoculation of the fungus. Using species of fungi from six different genera for systemic inoculation, Mankowski and Littleton[11] found that mice which received cortisone had much shorter survival times than the untreated controls.

Seligman[9,12] and Dukes and Tettenbaum[13] showed that chlortetracycline and oxytetracycline mixed with the intraperitoneal inoculum (the latter authors found the same effects with tetracycline also) markedly potentiated the pathogenicity of Candida albicans for white mice. Neither of these observers was able to induce this potentiation of infection with penicillin, streptomycin, or chloramphenicol. Bacitracin and sulfacetamide also had no potentiating effect in Seligman's experiments.

The purpose of this inquiry was, therefore, to determine whether the local use of cortisone and antibiotics, either alone or in combination, would facilitate fungus infections of the cornea.

One of the fungi used in these experiments was obtained from a case of fungus keratitis, one from a case of chronic onychomycosis, and the rest from autopsy material.

PART I. CORTISONE

A. IMMATURE RABBITS

EXPERIMENT *I. Aspergillus terreus applied to open corneal wounds.*

Eight immature rabbits* ranging in weight from 1,800 to 2,200 gm. were given bilateral standardized central lamellar corneal keratectomies while under sodium pentobarbital anesthesia. A two-mm. trephine with a guard set at 0.2 mm. was used and the button was dissected free. Before each keratectomy the trephine blade was dipped into

* From the Department of Ophthalmology and the Oscar Johnson Institute, Washington University School of Medicine.
† Trainee: National Institute of Neurological Diseases and Blindness.

* All rabbits used in this series of experiments were albino males obtained from Haskins Rabbitry, Saint Louis, Missouri.

a gross suspension of Aspergillus terreus* prepared by emulsifying the surface of a sporulating culture on Sabouraud's medium. Following the keratectomy, a drop of the fungus suspension was placed in each conjunctival sac. Aspergillus terreus which is of relatively low pathogenicity was chosen in preference to the more virulent species of Aspergillus in order that a minimal lesion might be produced in the untreated control eyes for comparison. Any organism which was very actively pathogenic would tend to produce so violent a reaction as to preclude comparison between treated and control eyes. The animals were divided into two groups of four each, using the left eye as control in both groups.

GROUP I (Cortisone): *What is the effect of local cortisone on a fungus contaminated wound?*

In the right eye approximately 1.5 mg. of cortisone acetate (Merck) was injected subconjunctivally just posterior to the superior margin of the limbus. This subconjunctival cortisone deposit was made initially at the time of fungus inoculation and then twice weekly until the animal was killed.

Cultures of the conjunctival sac on Sabouraud's and blood agar media were made prior to inoculation and at one and two weeks.

At the end of one week, three of the four animals showed conjunctival injection and corneal infiltrate in the right eye. By 10 days there was gross hypopyon in the same three cortisone treated eyes. These animals were killed at two weeks. All the left eyes showed transient iritis for three to four days and then cleared with only a small residual central scar of the keratectomy. The corneas of both eyes of the fourth animal remained clear despite an additional two weeks of cortisone therapy. Figures 1 and 2 are examples of the gross and microscopic appearance of the three infected eyes and controls.

GROUP II (Cortisone and tetracycline): *What is the effect of local cortisone in combination with a broad spectrum antibiotic on a fungus contaminated corneal wound?*

Subconjunctival injection of cortisone was made twice daily in the right eye only as in Group I, and, in addition, these four animals received conjunctival instillation of a suspension of 1.0-percent tetracycline and 1.5-percent hydrocortisone in sesame oil† three times daily. In these eyes the lesions developed a little more slowly. At the end of a week there was only a moderate iritis in the affected eyes, and at the end of 10 days there was a deep infiltrate and beginning hypopyon in three of the four right eyes. Although the fourth animal was treated for an additional two weeks, no lesion developed. All control eyes remained clear save for a small central scar. The gross and microscopic appearance of the infected eyes in Group II was identical with those of Group I.

All eyes were fixed in 10-percent formalin and mounted in paraffin. Sections were stained with Hotchkiss-McManus periodic-acid Schiff stain. All of the eyes that developed hypopyon ulcers on either subconjunctival cortisone or cortisone plus tetracycline were characterized by filamentous fungal infiltrate through the corneal stroma with aggregates of pseudomycelia lying on the distal side of Descemet's membrane and scattered hyphae in the anterior chamber inflammatory exudate. In some areas hyphae appeared to be penetrating intact Descemet's membrane.

The corneal stroma was markedly edematous and showed a generalized fibroblastic response but minimal vascularization except near the limbus. Inflammatory cell infiltration was of irregular distribution and heaviest in association with small areas of stromal necrosis.

* This fungus culture was kindly provided by Morris Moore, Ph.D., who also gave invaluable advice for these fungus studies.

† Supplied by Lederle Laboratories Division, American Cyanamid Company.

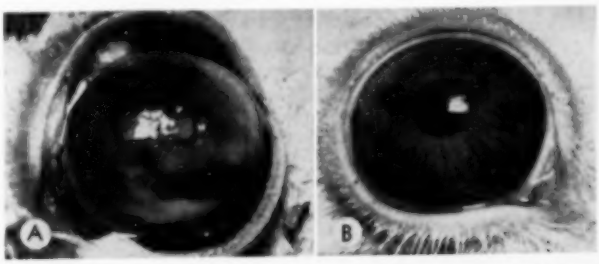

Fig. 1 (Ley). Experiment I, Group I: Aspergillus terreus with cortisone. (A) Cortisone-treated eye at 10 days, showing the marked corneal edema evident at the site of keratectomy, the stromal infiltration and hypopyon. (B) Control eye from the same animal at 10 days. Note the complete absence of inflammatory reaction.

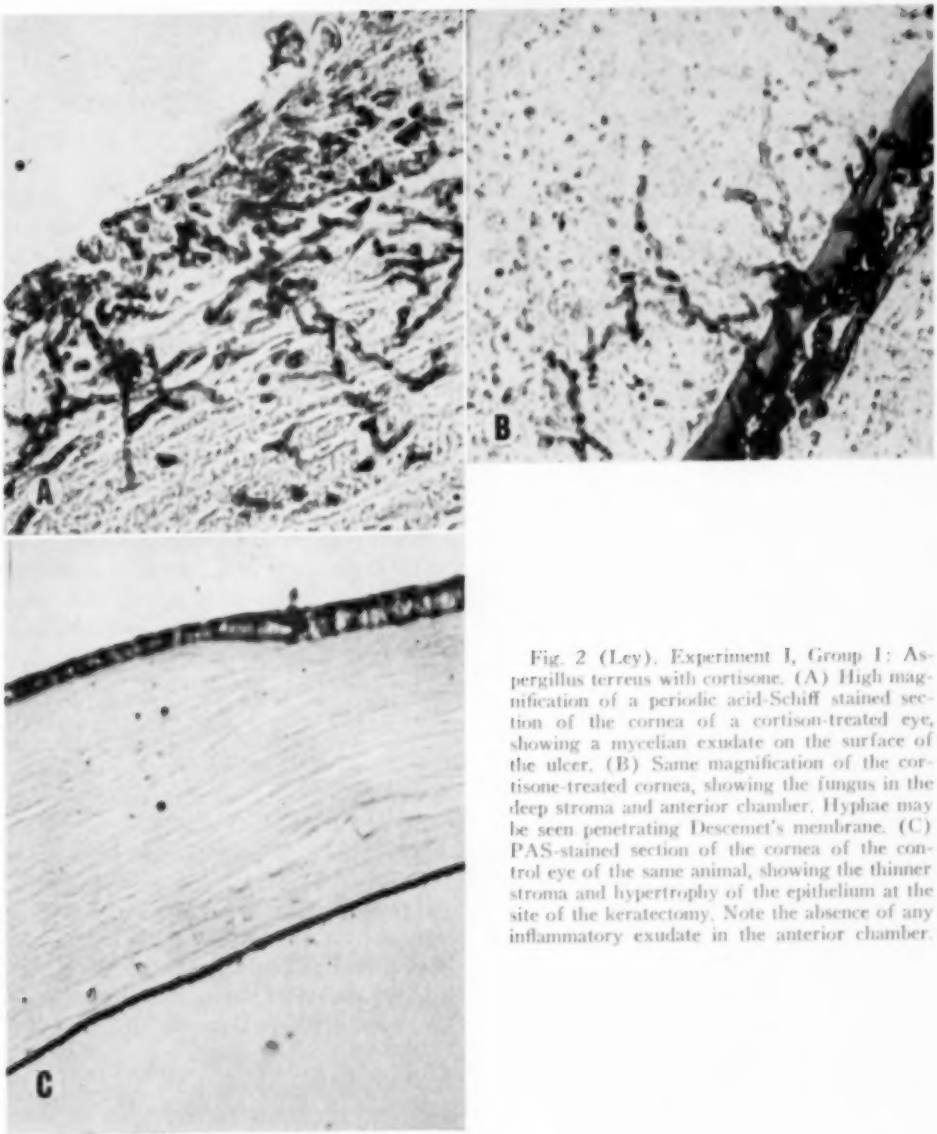

Fig. 2 (Ley). Experiment I, Group I: Aspergillus terreus with cortisone. (A) High magnification of a periodic acid-Schiff stained section of the cornea of a cortison-treated eye, showing a mycelian exudate on the surface of the ulcer. (B) Same magnification of the cortisone-treated cornea, showing the fungus in the deep stroma and anterior chamber. Hyphae may be seen penetrating Descemet's membrane. (C) PAS-stained section of the cornea of the control eye of the same animal, showing the thinner stroma and hypertrophy of the epithelium at the site of the keratectomy. Note the absence of any inflammatory exudate in the anterior chamber.

TABLE 1

EXPERIMENT I: *Aspergillus terreus* (surface inoculated)

Animal Number	Terminal Appearance		Microscopic Sections		Fungus Cultures
	Treated Eyes	Controls	Treated Eyes	Controls	
Group I—*Cortisone Treated (4 animals)*					
102	Opaque infiltrate	Clear	Fungus keratitis	Clear	+
105	Clear	Clear	Clear	Clear	—
108	Hypopyon ulcer	Clear	Fungus keratitis	Clear	+
109	Hypopyon ulcer	Clear	Fungus keratitis	Clear	+
Group II—*Cortisone and Tetracycline Treated (4 animals)*					
106	Hypopyon ulcer	Clear	Fungus keratitis	Clear	+
107	Opaque infiltrate	Clear	Fungus keratitis	Clear	+
110	Hypopyon ulcer	Clear	Fungus keratitis	Clear	—
112	Clear	Clear	Clear	Clear	—
TOTAL	6 infected	0			

The anterior chambers were filled with a dense inflammatory cell exudate on a heavy fibrin network. The free fungus filaments in the anterior chamber were surrounded with clumps of leukocytes, especially polymorphonuclears, and fibrin. With but one exception, the inflammatory response was confined to the anterior segment of the eye. All but one of the infected eyes had mycelian exudates on the surface of the corneas.*

The control eyes showed only epithelial proliferation and thinning of the corneal stroma at the site of keratectomy and the anterior chambers were free of exudate. No fungi could be demonstrated. The uninfected right eyes presented the same microscopic picture as the controls.

Cultures on Sabouraud's medium grew Aspergillus terreus from all the infected eyes with but one exception in which cultures were sterile. This sterile culture may be attributed to the use of cotton swabs for collecting cultures in the first experiment. The one section which showed fungus invasion of the lens and anterior vitreous was from an eye that cultured both Aspergillus terreus and fumigatus and the medium was quickly overgrown by the latter.

Table I summarizes the results of the first experiment in which it may be seen that six of the eight cortisone treated eyes developed fungus keratitis. In sharp contrast stand the controls which remained clear. No difference in incidence of infection was noted when topical tetracycline was used with cortisone.

EXPERIMENT II. *Aspergillus terreus injected into corneal stroma.*

What is the effect of local cortisone on deeply inoculated fungi?

Eight young rabbits weighing from 1,240 to 1,900 gm. were given 0.01 ml. superficial intralamellar injections of a gross suspension of Aspergillus terreus. The right eyes were treated with subconjunctival injections of cortisone acetate (Merck) approximately 1.5 mg. at the time of inoculation and thrice weekly. At the end of a week only one right eye showed infiltrate and hypopyon. All the remaining animals were reinoculated with the fungus suspension. One animal died before completion of the experiment. Three additional right eyes and one control eye developed gross ulcers with hypopyon and one right eye showed a dense stromal infiltrate. The microscopic findings in all the grossly infected eyes were comparable with those in Experiment I.*

* In three of the sections, Goodpasture-Gram stain demonstrated what appeared to be cocci; however, the fungus was the predominant organism.

* In one of the sections a considerable number of cocci were demonstrated by Goodpasture-Gram stain.

TABLE 2

EXPERIMENT II: *Aspergillus terreus* (intralamellar inoculated; 8 animals)

Animal Number	Terminal Appearance		Microscopic Sections		Cultures	
	Cortisone Treated	Controls	Cortisone Treated	Controls	O.D.	O.S.
41	Hypopyon ulcer	Clear	Fungus keratitis	Clear	+	−
42	Clear	Clear	Clear	Clear	+	−
43	Deep infiltrate	Clear	Fungus keratitis	Clear	+	+
44	Hypopyon ulcer	Hypopyon ulcer	Fungus keratitis	Fungus keratitis	−	+
45	Clear	Clear	No sections		+	−
46	Hypopyon ulcer	Clear	Fungus keratitis	Clear	−	−
47	(Died)					
48	Hypopyon ulcer	Clear	Fungus keratitis	Clear	+	−
TOTAL	5 infected	1				

The results of Experiment II are summarized in Table 2 in which it may be seen that five of eight cortisone treated eyes developed fungus keratitis as contrasted to only one control infection.

Pretreatment with cortisone for one week was required before a take could be secured in four of the treated eyes. It must be suspected that some attenuation of this fungus may have occurred in the five months that elapsed between Experiments I and II.

It should be pointed out that the experiments are not arranged in chronological sequence, but are grouped according to type of treatment, animal age, and organisms.

B. MATURE RABBITS

EXPERIMENT III. *What is the effect of local cortisone on corneal wounds contaminated with various species of fungi?*

Thirty-four mature rabbits of weights ranging from 3.0 to 4.5 kg. were subjected to bilateral two-mm. lamellar keratectomies and divided into two groups. (These animals had been used for tonography work and had been in the laboratory six months.)

GROUP I: *Aspergillus terreus*

The trephine wounds of 10 animals were inoculated with Aspergillus terreus and subconjunctival cortisone acetate (Schering) approximately 1.5 mg. was given with the inoculum and twice weekly thereafter for a total of five injections. Although the animals were observed for two weeks following the last injection (four weeks total), no infections developed in either treated or control eyes.

GROUP II: *Cephalosporium*

The trephine wounds of eight animals were inoculated with a species of Cephalosporium (Hyalopus)* and subconjunctival cortisone acetate was given as in the first group. None of the animals observed over the same period of time developed infections in the corneal wounds.

GROUP III: *Aspergillus fumigatus*

The corneal wounds of four animals were inoculated with a suspension of Aspergillus fumigatus and the right eyes were treated with subconjunctival cortisone acetate (Schering) approximately 1.5 mg. thrice weekly. Within 10 days three of the four right eyes developed deep hypopyon ulcers covered with pseudomembranes. One right eye and two of the four left eyes remained clear. Of the two infected control eyes, one perforated and one showed an area of ulceration one half the diameter of the right eye. Representative sections showed typical mycelian exudates on the surface of grossly infected anterior segments.

* This fungus was isolated from an active fungus keratitis and kindly provided by Dr. Ocasio Cabanas, Veterans Administration Hospital, Saint Louis, Missouri.

Group IV: *Candida albicans*

The corneal wounds of four animals were inoculated with a suspension of Candida albicans and the right eyes were treated with subconjunctival cortisone acetate (Schering) approximately 1.5 mg. thrice weekly. One animal died before completion of the experiment. Of the three remaining right eyes, two had hypopyon ulcers and one a deep infiltrate. The ulcers had a deep punched-out appearance and were accompanied by a thin white conjunctival discharge. Two control eyes remained clear and one ulcerated. Representative sections showed overwhelming fungus infestation of the anterior segment of the infected eyes with many yeast forms and pseudomycelia. Uninvolved controls showed no organisms.*

Group V: *Allescheria boydii*

The corneal wounds of four animals were inoculated with a suspension of Allescheria boydii (called Monosporium apiospermum in the asexual phase). The right eyes were treated with subconjunctival injections of cortisone acetate (Merck) approximately 1.5 mg. at the time of inoculation and thrice weekly for a total of six injections. At two weeks, three treated eyes showed deep corneal infiltrates, one with hypopyon. One of the control eyes had developed a small infiltrate one half the size of the corresponding treated eye and without hypopyon. One of the right eyes and three of the controls developed no infection. It is of interest to note here that only one of the four infected corneas had an ulcer, the other three corneal wounds having completely epithelized by the end of two weeks despite the presence of active infection. This correlated with sterile cultures from corneal scrapings in one of the actively infected animals.

The microscopic sections of the infected eyes were notable for deep corneal infiltration by hyphae with the typical morphology of Monosporium apiospermum, and localized areas of stromal necrosis with corresponding patches of dense cellular exudate (mostly polymorphonuclears) on the posterior corneal surface. Pseudomycelia, some with typical terminal bulbous conidia, were found along portions of the distal side of Descemet's membrane; whereas, in the anterior chamber yeast forms and spherules were predominant. Fungus filaments appeared to be penetrating intact Descemet's membrane. Figure 3 gives an example of the typical microscopic findings.

Group VI: *Geotrichum*

A suspension of a species of Geotrichum was inoculated into the standardized corneal wounds of four rabbits. The right eyes were treated with subconjunctival injections of cortisone acetate (Merck) approximately 1.5 mg. at the time of inoculation and thrice weekly for a total of four injections. No infections occurred in a 10-day period of observation.

Table 3 summarizes Experiment III in which it is observed that Aspergillus terreus, Geotrichum, and Cephalosporium did not produce fungus infections in the older animals despite the concomitant application of cortisone. Candida albicans, Aspergillus fumigatus, and Allescheria boydii all produced active lesions each of which was distinguished by a rather characteristic gross and microscopic picture. Although lesser pathogens did not produce any lesions, neverthless, when a fungus of sufficient virulence was used the depressant effect of cortisone on host resistance became apparent.

*An interesting demonstration of the comparative efficacies of culture techniques was made in conjunction with Groups III and IV of this experiment. Four grossly infected animals, two with Aspergillus and two with Candida, were selected. First, a cotton swab was used to collect inoculum from the surface of the ulcers which was then plated directly to one half of a Sabouraud's agar plate; secondly, the other half of the same plate was inoculated directly with a scraping of the corneal ulcer taken with a metal spatula. The results are summarized in Table 3A.

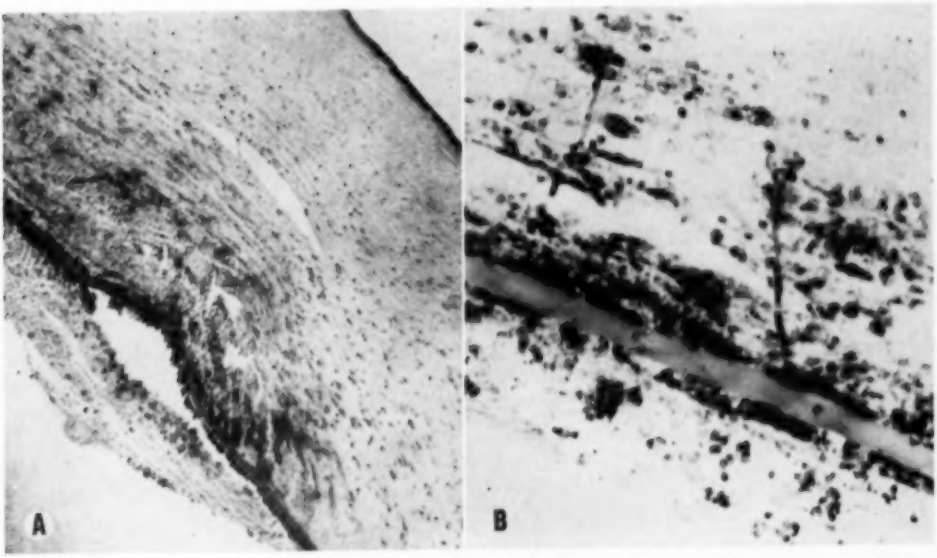

Fig. 3 (Ley). Experiment III, Group V: Allescheria boydii with cortisone. (A) Low magnification of PAS-stained section, showing localized deep corneal abscess with minimal involvement of the anterior stroma and no ulceration. (B) Higher magnification of the same section. Pointing upward in the stroma above Descemet's membrane are fungus filaments with characteristic terminal bulbous conidia typical of Allescheria boydii.

Part II. Antibiotics

Experiment IV. *(Aspergillus terreus). What is the effect of topical tetracycline on a fungus-contaminated corneal wound?*

Four immature animals ranging in weight from 1,800 to 2,000 gm. were given standard corneal keratectomies and the corneal wounds were inoculated with a suspension of Aspergillus terreus. The animals received topical applications of 1.0-percent tetracycline suspension in sesame oil* three times daily in the right eye. Within three days one rabbit developed a violent hypopyon ulcer and the cornea became completely opaque and vascularized by the end of two weeks. The remaining three did not show any sign of keratitis after four weeks of topical tetracycline therapy. All the controls remained clear save for a small central scar.

The eye that developed a keratitis while receiving topical tetracycline showed neither fungi with periodic acid Schiff stain nor bacteria with Goodpasture-Gram stain. In contrast to the cortisone treated specimens, this eye showed marked fibrovascular proliferation quite comparable to granulation tissue, indicative that, whatever the infectious agent, the eye had overcome it and was well on with the reparative process at the time of enucleation. Although this eye grew only Aspergillus terreus on culture, this keratitis must remain unclassified inasmuch as the fungus could not be demonstrated in the tissue sections. It must be concluded that tetracycline did not potentiate Aspergillus terreus infections.

Experiment V. *What is the effect of chlortetracycline when added to the intralamellar inoculum?*

Twelve immature rabbits ranging from 2,000 to 2,400 gm. were divided into two groups of six each.

* Supplied by Lederle Laboratories Division, American Cyanamid Company.

TABLE 3

EXPERIMENT III: (old animals)

Animal Number	Terminal Appearance		Cultures
	Cortisone Treated Eyes	Control Eyes	
Group I *Aspergillus terreus:* (10 animals; no lesions)			
Group II *Cephalosporium:* (8 animals; no lesions)			
Group III *Aspergillus fumigatus:* (4 animals)			
497	Hypopyon ulcer	Hypopyon ulcer	+
555	Ulcerated corneal infiltrate	Perforated ulcer	+
556	Hypopyon ulcer	Clear	+
557	Clear	Clear	not cultured
Group IV *Candida albicans:* (4 animals)			
565	Hypopyon ulcer	Clear	+
566	Corneal infiltrate	Clear	+
571	(Died)		
573	Hypopyon ulcer	Hypopyon ulcer	+

Animal Number	Terminal Appearance		Microscopic Sections		Cultures
	Cortisone Treated Eyes	Control Eyes	Treated	Controls	
Group V *Allescheria boydii:* (4 animals)					
574	Clear	Clear	Not sectioned		
576	Infiltrate and hypopyon	Small infiltrate *no* hypopyon	Fungus keratitis with anterior uveitis	Circumscribed fungus keratitis	+
578	Deep infiltrate	Clear	Fungus keratitis	Clear	–
580	Deep infiltrate	Clear	Fungus keratitis	Clear	+
Group VI *Geotrichum:* (4 animals; no lesions)					
TOTAL infected:	9	4			

GROUP I: *Allescheria boydii*

Into the right eyes an intralamellar injection of 0.01 ml. of a suspension of a culture of Allescheria boydii containing 2.5-percent chlortetracycline was made and into the left eyes a plain saline suspension of the same quantity of organisms was made. The right eyes developed a chemical keratitis with dense scar formation, but none of the eyes developed infections, nor were mycelia demonstrated in the globes that were sectioned.

GROUP II: *Aspergillus terreus*

Injections of a suspension of an Aspergillus terreus culture in 2.5-percent chlortetracycline were made into the superficial

TABLE 3A

COMPARATIVE CULTURES

Animal No.	Cotton Swab	Spatula Scraping
497	No growth	Asp. fumigatus Profuse growth
556	No growth	Asp. fumigatus Profuse growth
565	Candida albicans Profuse growth	Candida albicans Profuse growth
573	Candida albicans Very sparse growth	Candida albicans Profuse growth

corneal lamellae of the right eyes and the same number of organisms in plain saline were injected into the left eyes. No infections developed and the reactions and microscopic findings were the same as in Group I. Chlortetracycline did not potentiate Aspergillus terreus or Allescheria boydii.

EXPERIMENT VI *(Candida albicans). What is the effect of oxytetracycline when added to the intralamellar inoculum?*

Twelve young rabbits ranging from 1,500 to 2,200 gm. were given superficial intralamellar injections of 0.01 ml. of a suspension of Candida albicans containing 570,000 yeast forms per cu. mm. The suspension in the right eye contained 1.0-percent oxytetracycline, and in the left saline. One animal died the first day. All of the eyes became grossly infected. Within four days, nine of the 11 right eyes had ulcers 1.0 to 3.5-mm. larger in diameter than the controls. One was the same size as the control and one control was 2.0 mm. larger than the right. Eight of the right eyes and one of the left spontaneously perforated before enucleation at the end of one week

The microscopic sections showed massive anterior segment infections which differed only quantitatively. Cultures of all of the ulcers grew only Candida albicans with one exception in which there was a growth of B. subtilis in addition. Figure 4 gives an example of the gross and microscopic appearance of the infected eyes.

The results of Experiment VI are summarized in Table 4. It is evident that oxytetracycline strongly potentiated Candida albicans infections of the cornea.

DISCUSSION

Although the number of animals in any one experiment was small, the summation of the results falls into a significant pattern as shown in Table 5. (The experiments in which no lesions could be produced have not been included in this summary.) In all there were 26 animals in the groups in which fungus keratitis could be produced. Twenty of the cortisone-treated animals (approximately 80 percent) developed fungus keratitis and five of the controls (approximately 20 percent) developed fungus keratitis.

If one compares the results of experiments with mature rabbits and those with immature animals the contrast is striking. The adult animals had an incidence of 82 percent infected cortisone treated eyes and 36 percent infected control eyes. On the other hand, the immature animals showed 73 percent infected cortisone treated eyes but only seven percent infected control eyes. Part of this difference may be attributed to the fact that much more virulent organisms had to be used to produce infections in the mature animals. Nevertheless, it becomes evident that cortisone treatment resulted in from two to 10 times the incidence of fungus keratitis in the susceptible groups.

It should be pointed out here that in every experiment in which a lesion occurred in a control eye there was also a lesion in the fellow eye. Thus it is fair to assume that none of the isolated right eye lesions can be attributed to chance infection. This assumption would be invalid if in the group there was an isolated control eye lesion.

Since bacteria were also demonstrated in some of the sections prepared with Goodpasture-Gram stain, it might be argued that the fungi were present only as saprophytes; however, two facts refute this. First, once the keratitis became clinically manifest the blood agar cultures (with few exceptions) grew only fungi. Second, there was no decrease in the incidence of fungus keratitis when a broad spectrum antibiotic was used in conjunction with the cortisone.

It finally became apparent in the course of these experiments that older animals were resistant to the less virulent organisms. This was also the experience of Hervouet and Lenoir.[14] Using a species of Aspergillus freshly isolated from an active case of fungus keratitis, these authors were unable to produce lesions in corneas of large healthy rabbits, but by inoculating "small puny" ani-

Fig. 4 (Ley). Experiment VI: Candida albicans with oxytetracycline at one week. (A) Oxytetracycline-treated eye showing gross perforation with lens extruding. Note the marked purulent exudate. (B) Control eye from the same animal shows small deep central ulceration but is not perforated. (C) High magnification of deep corneal stroma and anterior chamber in an oxytetracycline-treated eye. Note the massive growth of monilial pseudomycelia.

TABLE 4

EXPERIMENT VI: *Candida albicans* + OXYTETRACYCLINE

Animal Number	Oxytetracycline Treated Eyes	Control Eyes
537	Perforated ulcer	1 mm. hypopyon ulcer
538	Perforated ulcer	4 mm. ulcer & deep keratitis
539	Perforated ulcer	3 mm. hypopyon ulcer
540	5 mm. hypopyon ulcer	2 mm. hypopyon ulcer
541	Perforated ulcer	2.5 mm. hypopyon ulcer
542	(Died)	
543	Perforated ulcer	2.5 mm. hypopyon ulcer
544	Perforated ulcer	2 mm. hypopyon ulcer
545	2 mm. hypopyon ulcer	Perforated ulcer
546	2 mm. hypopyon ulcer	2.5 mm. hypopyon ulcer
547	Perforated ulcer	3 mm. hypopyon ulcer
548	Perforated ulcer	2 mm. hypopyon ulcer
Totals: 11 pairs of eyes	8 perforated ulcers	1 perforated ulcer

mals they were able to reproduce the fungus keratitis. Certainly in this series, despite the use of cortisone, Aspergillus terreus infections could be produced only in young animals. Four of the five animals that developed fungus keratitis in Experiment II required a second inoculation of the fungus culture (or pre-treatment with cortisone) to produce a take—suggesting attenuation of the organism in the many transfers of the fungus culture in the five-month interval between these two experiments. Animal passage was not used in any of these experiments. All inocula were taken from Sabouraud's cultures.

Cephalosporium, although recently isolated from an active human fungus keratitis, could not be grown on mature rabbits' cornea. Bedell[15] reports a similar experience with Cephalosporium although he did not use cortisone. Stoewer[16] was also unable to grow on rabbit's cornea a freshly isolated species of fungus (identified only as a rose mold) from human keratitis, although it could be grown when inoculated into the anterior chamber and vitreous. A species of Geotrichum also did not grow on mature rabbits' corneas, despite use of cortisone.

TABLE 5

SUMMATION OF EXPERIMENTS WITH CORTISONE

	Number of Eyes with Fungus Keratitis	
	Cortisone	Controls
Young Animals		
Aspergillus terreus (8 animals)	6	0
Aspergillus terreus (7 animals)	5	1
Old Animals		
Aspergillus fumigatus (4 animals)	3	2
Candida albicans (3 animals)	3	1
Allescheria boydii (4 animals)	3	1
TOTAL ANIMALS: 26	20	5

Experiments I (Group II) and V (Group II) showed that neither topical tetracycline nor intrastromal chlortetracycline potentiated Aspergillus infections. The latter also did not influence the pathogenicity of Allescheria boydii. Candida albicans, on the other hand, provided a sharp contrast. Experiment VI demonstrated that oxytetracycline given with intrastromal Candida albicans produced much wider and deeper ulceration of the cornea than in the controls. Eight of the 11 treated eyes spontaneously perforated, contrasting with one perforation in the controls. That this is not simply due to the necrotizing effect of the oxytetracycline is suggested by the fact that in the experiment with Aspergillus terreus and with Allescheria boydii the concentration of chlortetracycline used produced dense scarring of the corneas without causing a single active fungus infection. It is obvious that further study of this phenomenon is required with the tetracycline group.

Summary and Conclusions

1. Cortisone applied to traumatized rabbit corneas in the presence of potentially pathogenic fungi resulted in an 80 percent average incidence of fungus keratitis in cortisone treated animals as contrasted with a 20 percent average incidence in the controls.

2. Mature animals required the use of more virulent organisms to produce lesions and had a higher incidence of infection in the control eyes. The spread between immature and mature animals was considerable. The cortisone treated younger animals showed a greater increase in the incidence of fungus keratitis over their controls than did the older animals.

3. A broad spectrum antibiotic used topically in conjunction with local cortisone treatment did not result in lower incidence of fungus keratitis.

4. Oxytetracycline combined with the inoculum potentiated Candida albicans infection of the immature rabbit's cornea.

640 South Kingshighway (10).

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Discussion

Dr. James H. Allen (New Orleans, Louisiana): I wish to thank the author for the privilege of reading his paper before the meeting and, in addition, for seeing some of the excellent sections from the experimental material. I also want to congratulate the author on presenting, for the first time, experimental evidence of activation of fungus infections of the eye by antibiotics and by steriods. The 4:1 ratio makes a clear cut demonstration.

This situation previously has been demonstrated clearly in virus infections and acute bacterial infections both clinically and experimentally. How-

ever, from the clinical standpoint it is difficult to drive this point home, because not all infectious processes treated by steroids show this exacerbation. For that reason it is sometimes difficult to persuade the clinician that such a therapeutic procedure in acute bacterial infections, virus infections, and now in fungus infections, is neither logical nor safe. Thus the microbiologist and the experimental ophthalmologist still have a problem to settle. Why are some infections activated by steroid therapy and others not?

In the case of viruses, there is some evidence that resistance is lowered by steroid therapy, and in certain viruses that the virulence of the organism is increased by pretreatment of the organism with steroids. In bacterial infections there is definite evidence of lowered resistance of the individual animal or patient to the infectious process.

At a recent clinical meeting it was announced that some observers believed that small doses of steroids did not affect the resistance of the individual, and therefore were safe in the treatment of clinical infectious diseases. Unfortunately, the microbiological literature, which is quite complete in experimental studies on this aspect of steroid therapy, does not confirm these clinical observations; as a matter of fact, there is very definite evidence that even small doses of steroids reduce the resistance of animals in the normal state. As a matter of fact, bacteriologists very frequently pretreat animals with steroids in order to insure infections in making inoculations with organisms of relatively low pathogenicity.

I would like to point out another fact that has been observed for bacterial infections, at least, and here it is emphasized again for fungus infections, and that is that antibiotics may show stimulating activity on certain infectious organisms. So, we must be sure that the antibiotic that is chosen from a therapeutic standpoint is effective in controlling an infectious process, and we must be careful not to choose an antibiotic in the therapy of an infectious disease which is likely to stimulate activity in the organisms.

INTERLAMELLAR FROZEN-STORED CORNEAL HOMOGRAFTS IN RABBITS*

P. K. Basu,† M.B., and Hugh L. Ormsby, M.D.
Toronto, Ontario

In order to gain some knowledge of the behavior and fate of frozen-stored corneal homografts, a series of experiments were carried out in rabbits. Fresh corneal homografts were used as controls.

Newer methods of freezing and thawing, together with glycerine pretreatment of mammalian tissues prior to freezing, have helped to solve many problems in long-term tissue storage.[1-9] In the field of corneal transplantation, Eastcott and co-workers,[10] Billingham and Rycroft,[11] Iliff and co-workers,[12] and McNair and King,[13] have reported some success with frozen-stored corneal homografts in humans and in animals.

To avoid the operative and postoperative complications responsible for graft opacification following penetrating and lamellar grafts in animals, the interlamellar method of grafting was chosen.[14-16]

Storage of corneal tissue

The donor tissue was removed from the animal within one hour of its death and placed in glycerine solution for from one to 18 hours prior to freezing.

The tissues preserved consisted of (1) the whole eye, (2) the whole cornea, with one to two mm. of the surrounding sclera, and (3) the isolated cornea, full thickness or lamellar, some with epithelium and endothelium and some without. Preservation of the whole eye enhanced the subsequent cutting of lamellar or full thickness grafts.

The donor materials were kept singly in sterile bottles fitted with airtight screw caps, containing 15-percent glycerine in saline. In some instances, the concentration of the

* From the Department of Ophthalmology, Faculty of Medicine, University of Toronto.
† From the Department of Ophthalmology, Ramakrishna Mission Hospital, Vrindaban, U.P., India. Colombo Plan Fellow in Canada.

glycerine was changed to 10 or 20 percent and the saline replaced with Ringer's solution. Tissues were soaked in these media for from one to one and one-half hours at room temperatures, or from one to 18 hours at +4°C. prior to freezing.

After glycerine pretreatment, the bottles were tightly closed and sealed with paraffin wax. Some of the tissues were frozen in bottles containing glycerine, and some without. Freezing was carried out by placing the bottles in a carbon-dioxide-alcohol mixture at −79°C. for 30 minutes. The rate of freezing was controlled by regulating the addition of the powdered carbon-dioxide snow, using a low-temperature recording thermometer. A slower method of freezing was carried out by keeping the bottles containing the tissues in contact with carbon-dioxide snow. Tissues were stored for periods up to seven months.

Following storage, tissues were thawed rapidly by placing the bottles in a water bath at 37°C. or pouring sterile warm saline at 37°C. into the bottle. Cultures were taken from these thawed tissues to ensure sterility.

The normal corneal consistency and transparency were found to be present in almost all the preserved corneas irrespective of the methods of freezing, thawing, or glycerine pretreatment, the length of storage, and type of the tissue stored. In five percent of donor materials, loosening of epithelium, changes in the compactness of the tissue, and loss of transparency were noted. Histologically, these latter materials showed loss of epithelium, folds of Descemet's membrane, and separation of corneal lamellae. These may have been due to trauma during the various stages of freezing and storage, or to some individual variation in the tissues.

Procedure

1. In the first set of experiments, the conjunctiva was incised and dissected anteriorly up to the limbus. The cornea was split with a Tooke knife along the conjunctival level, similar to that of a sclero-corneal trephine operation. This method gave a uniform cleavage plane in cornea. By cutting more deeply into the sclera at the limbus, a different cleavage plane could be obtained. Corneal and limbal incisions were avoided in order to gain better protection of the interlamellar pocket by the conjunctival flap. The free lip was lifted with a pair of corneal forceps and the graft, held by a pair of fine nontoothed forceps, was inserted into the pocket at the desired site and depth of the host cornea. The graft was spread carefully in its bed to avoid folding. The conjunctival flap was stitched in place and antibiotic ointments were applied. In some animals, for comparative results, simultaneous graftings were done bilaterally with fresh and preserved grafts. The size of the grafts varied from four to eight mm. and were cut by a Franceschetti trephine.

2. In a second set of experiments, the effect of removal of the host corneal lamellae superficial to the clear interlamellar grafts, and the action of tears on such grafts, were investigated.

A trephine incision was made on the anterior corneal lamellae of the host, overlying the clear interlamellar grafts, both fresh and preserved, after a period of 15 to 45 days following the primary operation. The size of the tissue removed was smaller than that of the graft, so that there was sufficient interlamellar portion of the graft within the host corneal lamellae at the periphery to prevent any anterior displacement of the grafts from the bed. In order to destroy the epithelium over the grafts, in half of the cases, repeated curettage of the surface overlying the trephined area was performed. In the other half of the cases, the epithelium was allowed to heal without any disturbance. The growth of the epithelium was checked by staining with fluorescein and slitlamp examination.

3. In the third set of experiments, the

graft was placed in a plastic ring 0.75 mm. thick, having a diameter larger than that of the graft, allowing a free space around the graft in the interlamellar pocket. Donor corneas were full thickness, placing fresh material in one eye of the recipient and stored material in the other.

Results

1. In the first series of experiments, using fresh and stored donor cornea in interlamellar grafts, 85 percent of the stored grafts remained clear from the beginning, and 10 percent developed delayed opacities after periods of from one to three weeks. Of the latter group, six percent of the opacities cleared in from three to six weeks and four percent remained opaque. Five percent of the grafts were opaque from the beginning and showed no tendency to clear.

Thus in the total series, final clarity resulted in 91 percent of the grafts. The host tissue tolerated the grafts well in an observation period up to 10 months (table 1).

Transparency and consistency of the stored grafts after thawing were similar to those of fresh grafts in 95 percent of the materials. Low antigenicity of the stored grafts might be a factor in the higher proportion of clear grafts. It was not known whether the transparent grafts were viable or not.[11]

TABLE 1

INTERLAMELLAR FROZEN-STORED CORNEAL HOMOGRAFTS IN RABBITS

Interlamellar Grafts	Fresh Tissue (percentage)		Stored Tissue (percentage)	
Clear from the beginning	60		85	
Clear for 1–3 weeks, then opaque	30	80	10	91
(a) Cleared after 3–6 weeks	20		6	
(b) Opacity persisted	10		4	
		20		9
Opaque from beginning	10		5	

2. In the second series of experiments, in which the epithelium was removed over the graft, the importance of a proper epithelial covering over the exposed interlamellar grafts was conclusively proved. Whenever the covering epithelium was defective, the interlamellar grafts became edematous, opaque, and vascularized (table 2).

3. In the third series of experiments, in which the pressure of the host corneal lamellae was removed by placing the graft inside a plastic ring within the interlamellar space, the consistency and clearness of the frozen grafts were similar to those of fresh grafts. The percentage of clear grafts was the same as that of grafts in the first experiment. Thus the pressure of the lamellae was not a factor in the preservation of clarity in these tissues (table 3).

Discussion

In these experiments, the appearance of

TABLE 2

EFFECT OF DESTRUCTION OF EPITHELIUM OVER INTERLAMELLAR GRAFTS FOLLOWING REMOVAL OF ANTERIOR HOST LAMELLAE

	Fresh Tissue (percentage)	Stored Tissue (percentage)
Control (good epithelium)		
Clear grafts	75	80
Opaque grafts	25	20
Destroyed epithelium		
Clear grafts	0	5*
Opaque grafts	100	95

* The interlamellar unexposed portion of the graft remained clear in five percent of eyes.

TABLE 3

EFFECT OF REMOVAL OF PRESSURE OF THE HOST CORNEAL LAMELLAE FROM THE INTERLAMELLAR GRAFTS

Interlamellar Grafts	Fresh Tissue (percentage)	Stored Tissue (percentage)
Clear grafts	75	80
Opaque grafts	25	20

opacities was associated with a definite but variable amount of vascularization of the host cornea, and the onset, density, size, and number of opacities were proportional to the severity of the reaction in the host eye. These reactions were similar in stored and fresh grafts, and were thought to be due to antigen-antibody reactions.[17, 18, 20] Antigenically, frozen grafts were weaker than fresh grafts. This might be due to loss of viability in the graft cells,[19] or to alteration of some specific but unknown substance. If, on the other hand, we were to assume that antigenicity is due to viable cells,[19] then the 10 percent of grafts showing antigenicity must have had viable cells.

In the grafts which were opaque from the beginning, comprising five percent of the total series, one percent were due to excessive operative trauma and another two percent to folding of the graft. In two percent, donor cornea was opaque after thawing, and the graft taken from it failed to clear. We concluded that cornea without proper consistency or transparency should not be used for grafting purposes.

Summary

1. The behavior and fate of frozen-stored homografts were studied, using the interlamellar method of grafting. Control experiments were done with fresh donor material.

2. Rabbit corneal tissues were pretreated with glycerol, frozen slowly, and stored at low temperature up to a period of seven months.

3. Clarity of the graft showed no difference following variations in the methods or length of storage. The period of observation was from two to 10 months on the grafted eyes.

4. Eight-five percent of interlamellar frozen homografts were absolutely clear. In 10 percent, opacities developed after a latent period of clarity of not less than seven days. These opacities were thought to be due to an antigen-antibody reaction. In five percent, the grafts were completely opaque, mostly due to unsuitable donor material. In all cases the host tissue tolerated the grafts well. The antigenicity of the frozen grafts was weaker than that of fresh grafts.

5. Defective corneal tissue which had no proper consistency or clarity after thawing never cleared up after grafting.

6. Following removal of the overlying host corneal lamellae after the primary operation, when complete and rapid epithelization occurred over the graft, clarity was maintained, but in the absence of proper epithelization, all the grafts became cloudy, vascularized, and fibrotic.

7. Interlamellar grafts, subjected to the action of the intracorneal fluid only, without any compression from the host lamellae, remained clear.

University of Toronto.

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Discussion

Dr. William Havener (Columbus, Ohio): At the present time the term "eye-bank" is a misnomer, and refers merely to an efficiently organized transportation and scheduling system. Such a system does work adequately, and any general hospital can easily supply its staff with an ample number of fresh corneas. Development of a method of adequate storage would make an eye-bank truly a bank, would facilitate transportation of corneas, and would remove the urgency in scheduling.

The glycerol freezing method appears to be the most promising type of preservation; indeed, tissue-culture methods have demonstrated growth viability of cells so preserved. These studies by Dr. Basu and Dr. Ormsby certainly seem to warrant clinical trials of transplantation of glycerine-frozen donor material. In fact, the superior results reported with frozen corneas could foretell the time when use of fresh corneas may be contraindicated.

The concept of an antigenic cause of late opacities is attractive and is supported by time relationships and by response to steroid therapy. By using Freund's adjuvant techniques we have recently succeeded in producing demonstrable antibodies to rabbit corneas in rabbits, thereby directly supporting the concept of antigenic causation of late opacity.

Finally, the clinical importance of always using clear donor material is borne out by these experiments. Under present conditions of corneal supply, a transplant should be done only when perfectly clear donor material is available.

I thank Dr. Basu and Dr. Ormsby for the opportunity of discussing their fine paper.

Dr. Harry King, Jr. (Washington, D.C.): My brief discussion of this paper is from the clinical standpoint.

Certainly this method of preservation has offered a notable advance in the ability to store corneas. However, there are certain disadvantages. It requires the maintenance of low temperatures, special care, and specialized equipment. These pose problems if one desires to transport corneas or store them for long periods.

At the time Eastcott and his co-workers first brought out this technique, in 1954, we were working on another method of storing corneas, that is, by dehydration in glycerine, without freezing. We felt that the replacement of glycerine in the tissues was the important factor, in preservation rather than the effect of freezing. Animal experiments proved that corneas dehydrated in glycerine remained viable, or at least they were uniformly successful in all lamellar grafts.

Since that time we have applied these preserved dehydrated corneas, stored with no refrigeration, to 10 human patients as lamellar grafts and these too have been successful. We have had the opportunity of using only one for a penetrating graft, because of the medicolegal aspects of this type of experiment, and this technique requires further studies.

I should like to propose, however, that eye-banks employ some method of corneal preservation. The technique outlined this afternoon, and the method that we have developed, are both successful for nonpenetrating grafts. Fresh corneal tissues, from eyes which are enucleated within 24 hours, should

be reserved by eye-banks for use in penetrating keratoplasty. Then, if an eye is not called for within 24 hours, that cornea can be dehydrated in glycerine and stored indefinitely without refrigeration, or it can be frozen after pretreatment in glycerine, as described today, and stored with refrigeration, to be used only for lamellar keratoplasty. This would result in the conservation of corneal tissue and a more constant supply.

DR. A. EDWARD MAUMENEE (Baltimore): I would like to add one word of warning about the use of preserved frozen material. It has been adequately shown that the cells in the cornea can be kept alive with the glycerine freezing technique. However, there is another factor of importance in the use of preserved donor material, that is, the lamellae tend to lose their adherence to one another. Thus, the donor cornea will become markedly swollen after transplantation. This is particularly true when the donor material is exposed to aqueous, as in penetrating grafts. It is for this reason that frozen preserved material is not satisfactory for penetrating grafts but can occasionally be used for lamellar transplantation.

I would also like to mention the use of interlamellar corneal grafts and the possible interpretation of these results as far as lamellar or penetrating grafts are concerned. Interlamellar grafts do not respond in the same way that lamellar or penetrating grafts do. Thus, an acellular piece of donor corneal material can be inserted into a interlamellar pocket and will remain clear for many months. Whereas, a similar piece of material would sluff or become cloudy with the other types of transplantation. Also, heterografts will remain clear in an interlamellar pocket without causing a reaction in the recipient animal; but in penetrating or lamellar grafts such material would cause a violent reaction in the cornea. Thus, I do not think the results obtained with interlamellar transplantation can be transferred to penetrating or lamellar grafts.

The interlamellar technique appeared so successful in experimental animals when we used it some five years ago that we thought this method of operation might be of value in patients whose corneas were unfavorable for grafting. We tried the technique in six patients but unfortunately all of the grafts became opaque.

OBSERVATIONS ON EXPERIMENTAL TRACHOMA AND INCLUSION CONJUNCTIVITIS*

PHILLIPS THYGESON, M.D., AND T. T. CROCKER, M.D.
San Francisco, California

Research on trachoma and inclusion conjunctivitis has been greatly retarded by the lack of a suitable experimental animal. Only monkeys and apes have so far been shown to be susceptible to these two related diseases, and their susceptibility has been definitely of a low order.

Inclusion conjunctivitis produces the more severe of the two experimental diseases and resembles the adult form of the human disease, particularly when it develops in the baboon; but experimental trachoma has been consistently a mild, self-limited, follicular conjunctivitis, lacking the four principal diagnostic features of trachoma, that is, (1) cytoplasmic inclusion bodies in epithelial cells, (2) follicles on the upper tarsus, (3) conjunctival cicatrization, and (4) pannus.

Although the epithelial-cell inclusions characteristic of human trachoma have not been found in experimental trachoma of the monkey, it must be remembered that Haberstaedter and Prowazek[1] first recognized them in the experimental disease of the ape. A further complicating factor in all experimental work with monkeys and apes has been their susceptibility to a so-called "spontaneous folliculosis."

The present study was undertaken as part of a long-term viral keratoconjunctivitis project in an effort to find a reliable means of diagnosing experimental trachoma and inclusion conjunctivitis and of differentiating them from spontaneous folliculosis. The

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work has been conducted along the following lines:

1. An attempt has been made to define minutely the clinical characteristics of experimental trachoma and experimental inclusion conjunctivitis so as to determine the existence of any features, discernible by gross or biomicroscopic examination, which would serve to differentiate one experimental disease from the other, and both from spontaneous folliculosis.

2. An attempt has been made by means of cortisone and hydrocortisone to increase the severity of experimental trachoma in the hope that pannus would develop, scars would form, and inclusions would become readily demonstrable.

3. An attempt has been made to discover cytologic changes of diagnostic significance by the examination of (a) epithelial scrapings, (b) expressed follicular materials, and (c) sections of excised tissues.

In previously reported studies,[2] baboons and monkeys were used extensively in work on these two diseases. Because of their availability, rhesus and cynomolgus monkeys have been used exclusively in the present study.

Clinical characteristics of experimental inclusion conjunctivitis

In the baboon, experimental inclusion conjunctivitis has appeared after an incubation period of from seven to 14 days as a subacute follicular conjunctivitis with ptosis of the upper lid (fig. 1) and a collection of exudate at the inner angle. The preauricular adenopathy that is frequently demonstrable in human cases has never been observed. Inclusion bodies have been found without too much difficulty, both in the incubation period and during the first few weeks of the clinical disease. Like the disease in humans, the principal area of follicular hypertrophy has been the lower fornix and lower tarsal conjunctiva, but the upper fornix has also been involved and occasionally a few follicles have encroached on the tarsal plate. The disease

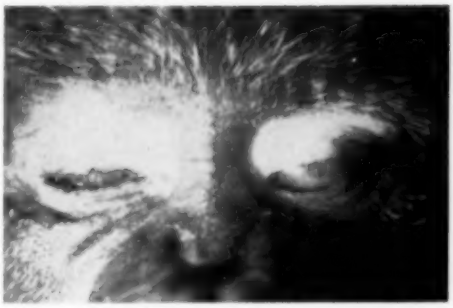

Fig. 1 (Thygeson and Crocker). Experimental inclusion conjunctivitis in the baboon, showing ptosis and conjunctival exudate.

has been self-limited, lasting usually several months and healing without any residual changes in the conjunctiva.

In the rhesus and cynomolgus monkeys the experimental disease has been milder than in the baboon. Onset has been insidious and there has been no ptosis or noticeable accumulation of conjunctival exudate at the angles. As would be expected, it has been more difficult to find inclusion bodies in scrapings from this milder disease, but diligent search has always been rewarding (fig. 2). In all other respects, including areas of involvement, duration, susceptibility to reinfection, and so forth, the disease in these animals has been identical with that previously observed in the baboon.

The biomicroscope has been used for the examination of these animals without contributing anything to the clinical picture. The cornea has never shown any epithelial keratitis and there have been no limbal changes or extension of corneal vessels. Epithelial scrapings from the disease have contained, in addition to inclusions, a predominance of neutrophils in the early weeks of the disease; later the exudate has dwindled and has then contained a mixture of neutrophils and round cells. In scrapings taken weekly it was observed that the leucocytic reaction terminated several weeks to a month before the disappearance of the conjunctival follicles. Some time before the follicles disappeared there was a clearing of the infiltrate

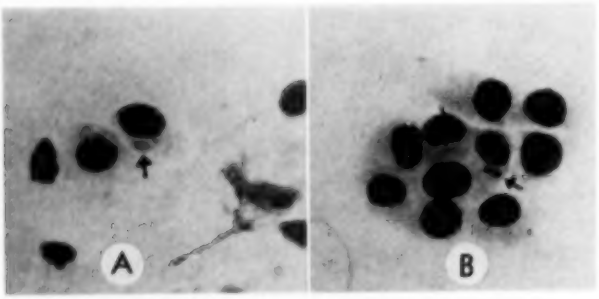

Fig. 2 (Thygeson and Crocker). Inclusion bodies in epithelial scrapings from experimental inclusion conjunctivitis in M. rhesus. (Giemsa stain. ×1,375.)

between the follicles so that in its last stages experimental inclusion conjunctivitis bore a resemblance to folliculosis, with its prominent follicles and absence of conjunctival infiltration.

There has been no difficulty in transmitting this disease from baboon to baboon or from monkey to monkey, and no resistance of any particular animal has been noted. When the experimental disease has cleared, reinfection has always been possible and the second infection has differed in no way from the first, indicating that not even a partial immunity has developed. Animals in which experimental inclusion conjunctivitis has followed its usual course have then been inoculated with scrapings from trachoma, and have developed typical experimental trachomatous disease. Experimental inclusion conjunctivitis has been transmitted in series through six animals and there seemed no reason to believe it could not have been transferred indefinitely if animals had been available.

The severity of the experimental disease has varied with the amount of the inoculum and the type of animal. In baboons receiving a heavy inoculum, a disease strictly comparable to the subacute follicular form of the human disease was obtained in a few instances (fig. 3). In the rhesus and cynomolgus monkeys, however, the onset has always been insidious, even when a heavy inoculum has been transferred directly from early, active human cases.

In summary, it may be said that in baboons it has been possible to produce an experimental inclusion conjunctivitis comparable to the adult form of the human disease and with all its salient characteristics, that is, inclusion body formation, pseudoptosis, and conjunctival follicular hypertrophy, with spontaneous healing after a period of several months. In rhesus and cynomolgus monkeys the experimental disease has differed from the human disease in having an insidious onset rather than an initial period of acute or subacute inflammation.

Clinical characteristics of experimental trachoma

In view of the much greater severity of trachoma than of inclusion conjunctivitis in

Fig. 3 (Thygeson and Crocker). Experimental inclusion conjunctivitis in the baboon, showing follicular hypertrophy of the conjunctiva.

man, it has been surprising to find that experimental inclusion conjunctivitis is much more severe than experimental trachoma. In our experience with inoculations of more than 50 animals of three different species, the onset of experimental trachoma has invariably been insidious—so insidious, in fact, that it has never been possible, as it has been with experimental inclusion conjunctivitis in the baboon, actually to name the day of onset. There has been some difference in the severity of the experimental disease (fig. 4) according to the amount of the inoculum, but in no instance have we produced a disease comparable to typical experimental inclusion conjunctivitis. Ptosis of the upper lid has never been observed, nor have inclusion bodies been demonstrated in conjunctival scrapings in spite of the most diligent search.

Nevertheless, experimental trachoma has resembled an atypically mild form of experimental inclusion conjunctivitis and has run the same, if somewhat more protracted, course to spontaneous healing. We have observed experimental trachoma in the monkey in which follicular hypertrophy persisted for as long as a year. Julianelle[3] reported an instance of the experimental disease in a rhesus monkey which was still active after three years but we never have seen an animal in which the disease lasted so long.

Fig. 4 (Thygeson and Crocker). Experimental trachoma in M. rhesus, showing follicles in upper fornix.

In the more severe cases there has been a small amount of exudate at the angles in which a predominance of neutrophils has been demonstrable in the early stages of the disease. Later, neutrophils and small round cells have been seen in approximately equal numbers. Careful biomicroscopic examinations of the conjunctiva, limbus, and cornea have revealed no lesions of diagnostic significance, and in no instance has there been the slightest evidence of corneal involvement or of any conjunctival scarring. In a few instances there has been encroachment of follicles onto the tarsal plate. Animals recovered from experimental trachoma have shown no increased resistance to subsequent reinfection and no resistance to infection with inclusion conjunctivitis.

In our experience it has been possible to produce experimental trachoma only by inoculation of the epithelial surface of the conjunctiva. Attempts to produce the disease by transmission of material through the skin of the lid to the subconjunctiva have been uniformly unsuccessful. For this reason we feel quite certain that the agent of the disease parasitizes the epithelium only, as is certainly the case in the human disease.

To summarize, it may be said that experimental trachoma bore little or no resemblance to human trachoma, differing principally in (1) its mild nature, (2) its failure to involve the tarsal plate and cornea, (3) the absence of conjunctival cicatrization, (4) the fact that it invariably heals spontaneously, and (5) the absence of inclusion bodies.

Biomicroscopic examination provided no diagnostic help. Experimental trachoma differed from experimental inclusion conjunctivitis principally in its more benign nature but longer clinical course, in the absence of inclusions, and in the absence of ptosis of the upper lid. It can be said that a differential diagnosis between experimental trachoma and experimental inclusion conjunctivitis is possible in their early stages but not possible

in their late stages, and that since inclusions have been found only in experimental inclusion conjunctivitis, the differentiation can be made most easily by microscopic examination of epithelial scrapings.

Characteristics of spontaneous folliculosis

Reports on spontaneous folliculosis in monkeys have come principally from the Giza Memorial Ophthalmic Laboratory when it was under the direction of Dr. Rowland P. Wilson[4] and Dr. John Bland.[5] In a note on trachoma research in the laboratory's Third Annual Report (1928), Wilson[4] called attention to a natural follicular disease of the conjunctiva in monkeys and apes which complicated the interpretation of the results of experimental inoculations of these animals with trachomatous materials. In the Fourth Annual Report (1929)[4] he described spontaneous folliculosis of monkeys as follows:

"The condition is characterized by the presence of hard, well-defined, semitranslucent follicles, especially at the outer and inner angles of the upper tarsus and also in both fornices; such follicles have never been observed to rupture spontaneously. They are always freely moveable over the subconjunctival tissue but never occur on the conjunctiva of the tarsus of the upper lid. These follicles may often be missed if the conjunctiva is not examined with a corneal loupe."

Bland[5] also urged strongly that spontaneous folliculosis be taken fully into consideration in all work on monkeys.

Our observations on spontaneous folliculosis in the United States have been limited to two cases only, since with these two exceptions our monkeys and baboons have been free of the disease. It is interesting in this connection that Julianelle,[3] in his very extensive work on experimental trachoma in rhesus monkeys, did not find spontaneous folliculosis a problem.

One of us (P. T.) observed the disease while working in Egypt at the Giza Laboratory with Wilson and was struck by its resemblance to mild forms of human folliculosis. It was not associated with conjunctival infiltration and the follicles therefore stood out more sharply than do the follicles in the follicular conjunctivitis of experimental trachoma and inclusion conjunctivitis. In our opinion it could only be confused with the experimental diseases in their healing stages.

In the two cases we have seen in this country, there has been no exudate and cytologic examination of epithelial scrapings has revealed nothing distinctive. Although our observations have thus not fully substantiated the fears expressed by Wilson and Bland, we do recognize this spontaneous disease as a definite hazard in experimental work on both trachoma and inclusion conjunctivis.

Effects of cortisone and hydrocortisone on experimental trachoma

Ormsby and associates[6] were the first to call attention to the activating effect of cortisone on inclusion conjunctivitis. Later Nataf and associates[7] and Thygeson[8] reported a similar effect of cortisone on certain cases of trachoma. It was felt, therefore, that a serious effort should be made to see if these steroid hormones could be used to increase the severity of the experimental disease and thus facilitate diagnosis, both clinically and by making possible the demonstration of inclusions or other significant cytologic changes.

In experiments designed to test this possibility, 12 animals infected with trachoma were injected subconjunctivally twice weekly for two weeks with cortisone or hydrocortisone. Although mild clinical activation occurred in three animals, there was no modification of the essential features of the experimental disease, no limbal or corneal changes developed, and no inclusion bodies could be demonstrated. We intend, however, to explore the possibility that a cortisone effect adequate for our purposes might de-

pend on high dosage or different modes of application.

Effects of cortisone and hydrocortisone on experimental inclusion conjunctivitis

Cortisone and hydrocortisone have also been administered twice weekly for two weeks by subconjunctival injection to three monkeys infected with inclusion conjunctivitis in attempts to increase the severity of the experimental disease, one animal receiving cortisone and two hydrocortisone. No major modification of the experimental disease occurred.

Cytologic changes in experimental trachoma

All attempts to demonstrate inclusion bodies in epithelial scrapings from experimental trachoma have failed, nor have we been able to identify any free elementary or initial bodies. Some of the cytologic changes characteristic of human trachoma[9] have been noted, however; that is, the presence of an occasional macrophage and plasma cell in the exudate, and a very definite neutrophilic reaction (fig. 5). A few bacterial forms have been observed but we felt they did not represent true secondary infection. The normal bacterial flora of the monkey conjunctiva is much more extensive than the flora of the human conjunctiva, and we feel that these bacteria were probably normal saprophytic forms. We never have seen a spontaneous bacterial conjunctivitis in the monkey, nor have we ever been able to infect the monkey conjunctiva with any of the ordinary conjunctival pathogens.

Cytologic changes in experimental inclusion conjunctivitis

The cytologic findings in epithelial scrapings from experimental inclusion conjunctivitis have been similar except that the leucocytic reaction has generally been more prominent and the macrophages have been more rare. As noted above, typical inclusions have been demonstrated fairly easily.

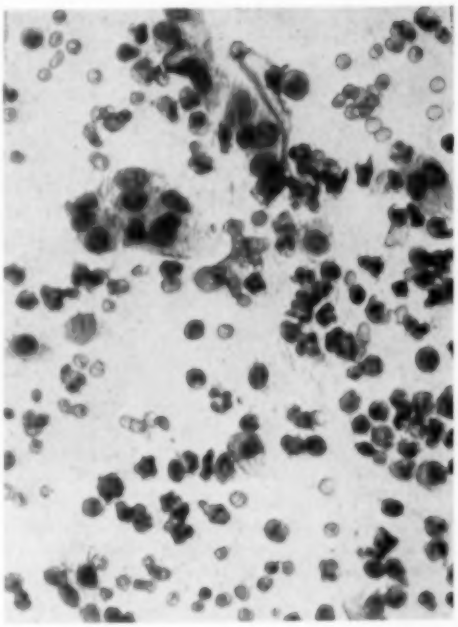

Fig. 5 (Thygeson and Crocker). Neutrophils in conjunctival scrapings from experimental trachoma of M. rhesus.

A study of follicular expressions, so useful in the cytologic diagnosis of human trachoma, had no value in the diagnosis of either experimental trachoma or experimental inclusion conjunctivitis. In both conditions the follicles have been hard, very difficult to express, and the contents when studied cytologically have revealed nothing of interest. In no instance were the degenerative changes of trachoma seen in preparations from either of the two experimental diseases. The study of biopsy specimens has been more rewarding. We feel that experimental trachoma and inclusion conjunctivitis can be differentiated from spontaneous folliculosis in most instances on the basis of the subepithelial infiltrate present in both experimental diseases (figs. 6 and 7) but never occurring in spontaneous folliculosis (fig. 8).

To summarize, then, it can be said that

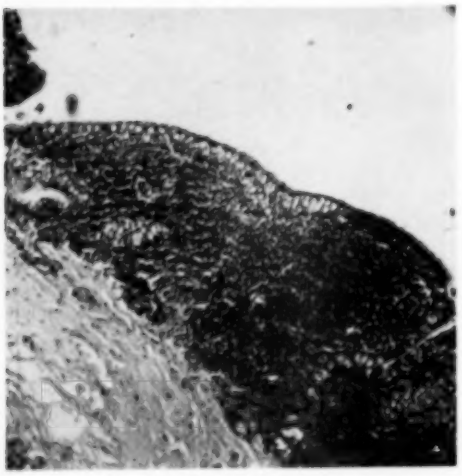

Fig. 6 (Thygeson and Crocker). Biopsy of conjunctiva from experimental inclusion conjunctivitis in M. rhesus, showing follicular conjunctivitis with infiltration between follicles. (Hematoxylin-eosin. ×200.)

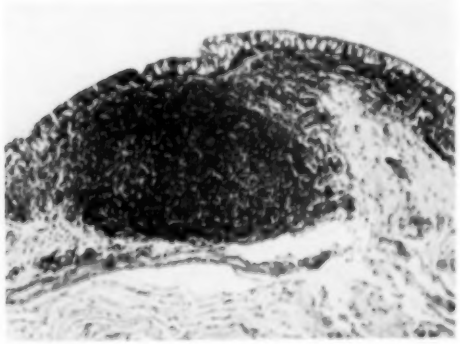

Fig. 7 (Thygeson and Crocker). Biopsy of conjunctiva from experimental trachoma in M. rhesus, showing follicular hypertrophy with infiltration between follicles. (Hematoxylin-eosin. ×200.)

experimental inclusion conjunctivitis can be diagnosed with certainty in the experimental animal, first because it resembles the human disease, and second because it displays the characteristic cytologic picture of the human disease. Experimental trachoma, on the other hand, bears little resemblance to human trachoma and reflects the cytologic picture of

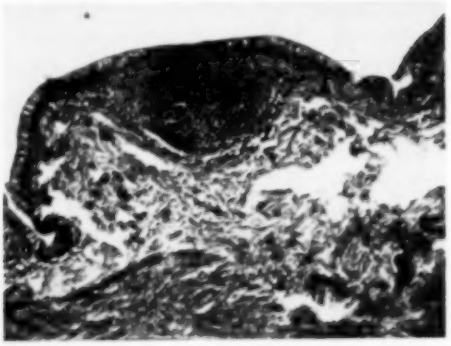

Fig. 8 (Thygeson and Crocker). Biopsy of conjunctiva from spontaneous folliculosis, showing isolated follicles with normal conjunctiva between follicles. (Hematoxylin-eosin. ×200.)

human trachoma in a very minor way only. From our limited observations on spontaneous folliculosis in monkeys, we feel that it can be differentiated reliably from experimental trachoma and experimental inclusion conjunctivitis except in their late stages.

Discussion

Animal experimentation as an investigative tool in trachoma research is certainly far from satisfactory. Our observations do not permit us to agree with Wilson's[10] statement that spontaneous folliculosis in the monkey is absolutely indistinguishable from the disease produced by the inoculation of trachomatous materials, but it is regrettably true that the existence of the spontaneous disease serves inevitably to cast doubt on conclusions based on animal experiments performed in the past when the possibility of this complication was not taken into consideration.

On the other hand, although experimental trachoma bears little resemblance to the human disease, its existence has been proven beyond doubt by Nicolle, Cuenod, and Blaizot,[11] and more recently by Bland.[12] This investigator transferred the simian trachoma from monkey to monkey and then to two human volunteers who developed typical trachoma with inclusions; the experimental

human disease was then transferred again to the monkey. Bland was unable to pass spontaneous folliculosis from animal to animal or to human volunteers.

Unsatisfactory as the situation is, no substitute for animal experimentation in trachoma seems likely to become available in the near future. The studies on the several types of conjunctivitis produced by viruses of the new APC (adenoido-pharyngo-conjunctival) group were all made without the aid of animal experimentation, but these viruses grew well in tissue culture and produced specific cytopathogenic changes in the tissue cells which could be specifically neutralized by antiserum. Unfortunately no such conditions prevail for work on trachoma virus. It thus behooves the trachoma investigator to use animals for experimentation when he must but to take care to use those free from spontaneous folliculosis. It need hardly be said that he can resort to the use of human volunteers only for the control of crucial experiments on this still dangerous disease.

Summary and Conclusions

Experimental trachoma and experimental inclusion conjunctivitis have been produced and studied in the baboon, the rhesus monkey, and the cynomolgus monkey. Experimental inclusion conjunctivitis is a characteristic clinical entity resembling in many respects the disease in man, but experimental trachoma is a mild disease having none of the diagnostic characteristics of human trachoma. Spontaneous folliculosis, in our experience not common in monkeys captive in the United States, can be differentiated from both diseases except in their late, healing stages.

The subconjunctival use of cortisone and hydrocortisone failed to modify significantly either of the experimental diseases. It had been hoped their severity would be increased as the severity of both human diseases has been increased by topical applications of these preparations.

The cell changes observed in epithelial scrapings from experimental inclusion conjunctivitis were the same as those found in the human disease, that is, cytoplasmic inclusion bodies and a predominance of neutrophils in the exudate. The cytologic picture in experimental trachoma, on the other hand, did not resemble that of the human disease and contained nothing of major diagnostic significance. The examination of expressed follicular material failed to yield diagnostic information. Biopsy examination was useful in differentiating the two experimental diseases from spontaneous folliculosis except in their late stages.

It is concluded that monkeys and baboons can be used with profit in the study of inclusion conjunctivitis but that, at the present time, conclusions based on monkey studies in trachoma must be confirmed by inoculation experiments in human volunteers.

220 Meridian Road (26).

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Discussion

Dr. Hugh L. Ormsby (Toronto): This paper marks another milestone in an almost life-long interest Dr. Thygeson has shown in the trachoma and inclusion conjunctivitis problem. This paper has such finality about it in Dr. Thygeson's hands, that one is left almost with a void in so far as discussion is concerned but there are certain points which I would like to bring out.

In the first place, the recent work on the APC viruses, using the HeLa cells, brought a new hope that the trachoma and inclusion conjunctivitis viruses might be grown in tissue culture. Dr. Snyder and his co-workers in Boston are following this method of investigation very vigorously. We too have followed along these lines in our own laboratory but over a period of three or four years have had no success in this way.

We have known for many years that trachoma and inclusion conjunctivitis could be transferred to monkeys, and it may well be that we have to go back to these animals to work out the problems of trachoma and inclusion conjunctivitis. Another point has been brought out in Dr. Thygeson's study—trachoma is a more highly specific virus for the human since it produces a more virulent disease in man than in animals.

I believe that we may be able to carry out the studies that we wish to do, with the inclusion conjunctivitis virus in animals and not worry too much about the trachoma virus. I don't know whether Dr. Thygeson agrees with this or not. If we can, we have a very good experimental animal in the monkey.

Now what are the immediate problems? First of all there will be many new antibiotics available as the years go by. Some of these antibiotics are already in use in these diseases, and some of the future antibiotics may be more effective. This laboratory animal will certainly help us differentiate good therapeutic agents from the bad and Dr. Thygeson indicates in his paper the technique which may be used in such studies. Secondly, the whole problem of steroid treatment in these diseases is certainly not yet final. Moreover, there will be many new steroids available. There is very little doubt that cortisone and hydrocortisone do exacerbate this disease in the human.

PERSISTENCE OF TOXOPLASMA GONDII IN OCULAR TISSUES: I*

Michael J. Hogan, M.D., Phyllis A. Zweigart, A.B., and Ann Lewis, A.B.
San Francisco, California

This report constitutes the first in a series of investigations concerning (1) the persistence of Toxoplasma gondii in ocular tissues of experimentally infected animals; (2) the suppressive and curative effect of various chemotherapeutic agents on the induced disease; and (3) the effect of steroids alone and in combination with chemotherapeutic agents on healed and inapparent toxoplasmic eye lesions. These studies are now in progress, and will be reported.

It has been established by a number of observers that Toxoplasma gondii will survive in the tissues of various animals for

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considerable periods of time. Using the RH strain, Jacobs[1] recovered viable organisms from pigeon brains up to 33 months after inoculation; Ruchman[2] isolated live toxoplasmas from rat brain two years after inoculation; Jacobs[3] found that the brains and livers of three out of four puppies harbored the viable parasites nine to 10 months after parenteral inoculation; and Weinman,[4] using a less virulent "w" strain of toxoplasma isolated by Wolf and Cowen from a human case, recovered the organisms from mouse brain nine to 11 months after inoculation.

These findings may be compared with Wilder's[5] observation of organisms resembling toxoplasma in human tissue sections of 53 cases of chorioretinal inflammation, and Jacobs'[6] isolation of the live organisms from a case of human adult chorioretinitis which recurred during a period of eight years. Two important questions are posed by these previously mentioned findings: (1) The length of time toxoplasma might persist in the ocular tissues of experimental animals, and (2) what clinical and laboratory evidence accompanies this infectious process.

Preliminary investigations designed to answer these questions were instituted, using two separate series of guinea pigs. This animal was selected becaue of its relative resistance to toxoplasmic infection, and because the size of the eye does not prohibit ophthalmoscopic examination. The serum of each animal was tested just prior to commencement of the experiment. Only those animals with negative methylene blue dye test titers at 1:16 were used in the following two experiments.

Experiment I

Fifty-four animals were inoculated in the vitreous of one or both eyes with the RH strain of toxoplasma. A total of 60 eyes were injected. The dosages varied from 1,000 to 6,000 live toxoplasmas which were obtained from the ascitic fluid of mice which had been infected three or four days previously. Ophthalmoscopic and serologic examinations were done at intervals following the injection.

Thirty-nine animals developed definite clinical evidence of acute chorioretinitis. The remaining 15 appeared normal, showed questionable lesions, or developed panophthalmitis, endophthalmitis, or cataracts. All animals developed significant dye test titers within one to two weeks after inoculation.

The eyes of these animals were enucleated at various times, ranging from less than one month to a year after inoculation. The eyes were divided and part of the chorioretinal tissue was ground and injected into mice. The remainder was reserved for histopathologic examination.

Toxoplasmas were recovered from the inoculated mice in 37 out of the 60 eyes. They were observed in tissue sections from 14 eyes. In four instances, the organisms were observed microscopically but were not recovered from mouse inoculation. Table 1 summarizes this study.

This pilot experiment demonstrated that it was possible to produce a chorioretinitis by intravitreal inoculation of toxoplasma. It also showed that the organisms could be recovered from the eye up to nine months after inoculation. In Experiment II it was decided

TABLE 1

Experiment I: Recovery or microscopic demonstration of Toxoplasma from guinea pig ocular tissue

Postinoculation time	Less than 4 wk.	4–8 wk.	8–16 wk.	5–8 mo.	9 mo.	12 mo.	Total
Number of recoveries	10	13	9	4	1	0	37
Number inoculated	15	16	13	6	8	2	60

to extend the observations to a two-year period, and to investigate the dye-test results more comprehensively.

Experiment II

Peritoneal fluid was withdrawn from mice inoculated three or four days previously with the RH strain. Using a standard Neubauer counting chamber and appropriate dilutions, a suspension containing approximately 5,000 toxoplasmas in 0.05 ml. of a 10-percent serum-saline solution was prepared. This amount was immediately introduced into the vitreous of the right eye of 60 guinea pigs.

Ophthalmoscopic examinations were done at frequent periods. By trial, it was learned that the optimum time for these examinations was three weeks after inoculation. At this time, the fundus usually could be seen clearly, and the presence or absence of inflammation could be determined. For the purpose of this experiment, further fundus examinations were considered unnecessary.

Serologic examinations were performed on the guinea pigs at monthly intervals. The dye test titers of all animals rose to 1:4,096 or higher.

Groups of these animals were killed at regular intervals after inoculation. The inoculated eyes were excised, the tissues aseptically ground in a mortar, and injected intraperitoneally into mice. Those mice which did not develop toxoplasmosis within three weeks were killed and the brains inoculated into fresh mice. If these mice in turn remained healthy, the procedure was repeated for an additional passage. (No further subpassages were attempted because in our extensive experimental work with this highly virulent RH strain, we have never recovered the organisms on subpassages.)

The previous experiment showed that the most reliable method of demonstrating organisms in the eye was by inoculation into mice. Therefore, microscopic sections were not done in this experiment.

Results

Two of the guinea pigs died three months after inoculation, apparently from pneumonia. Toxoplasmas were recovered from the eye of one animal. However, these two guinea pigs are not included in the following tables or discussion.

At the end of four months toxoplasmas were recovered from seven out of 10 guinea pig eyes; after six months from four out of 10 eyes; after eight months from three out of seven eyes; after 10 months from eight out of 10 eyes. The 21 additional guinea pigs which were inoculated are to be kept for two years before enucleation. The findings of these isolations are summarized in Table 2.

All but eight of the 37 animals developed unquestionable ophthalmoscopic evidence of an acute chorioretinitis. The eight exceptions comprised a group of three guinea pigs with cataracts; three with questionable chorioretinitis; one with a massive vitreous exudate; and one animal with no observable inflammation at all. Toxoplasmas were recovered from the eyes of three animals in this group (one that showed a cataract; the one with massive vitreous exudate; and the apparently negative one).

Within a month, 22 of the guinea pigs developed dye test titers of 1:16,384 or greater, and the remaining 13 showed titers

TABLE 2

Experiment II: Postinoculation recovery of Toxoplasma from guinea pig ocular tissue

Postinoculation time	4 mo.	6 mo.	8 mo.	10 mo.	Total
Number of recoveries	7	4	3	8	22
Number of animals studied	10	10	7	10	37

of 1:4,096. At the end of four months the titers were as follows: one animal, 1:16,384; 10, 1:1,024; 10, 1:256; two, 1:64; and two had dropped to 1:16.

After eight months, four of the animals had titers of 1:4,096; three were 1:1,024; and the rest were 1:256. Several of the titers were up one four-fold dilution from the preceding four months, and two of them were up an eight-fold dilution. At no time during the whole experiment did the animals appear ill. There was no correlation between the dye test titers and the frequency of recovery of organisms. Tables 3, 4, 5, and 6 summarize these findings.

Discussion

These experiments demonstrate a very nice method of production of a chorioretinitis in the eyes of guinea pigs by the injection of moderate numbers of toxoplasma organisms into the posterior segment of the eye. The inflammation so produced develops rather slowly and definite ophthalmoscopic evidence of its presence is apparent within one to three weeks. Heavy opacification of the vitreous is an early indicator of the development of chorioretinal foci. This experimental lesion is a very satisfactory one to study not only because of the ease of examination of the eyes, but because of the facility with which the animals are handled.

TABLE 3

Guinea pig enucleation at four months

Guinea Pig Number	DT* at 1–2 mo. Post-inoculation	DT at 4 mo.	Recovery of Organisms
1	1:4T†	1:4T	+
2	1:4T	1:1T	+
3	1:16T	1:1T	+
4	1:16T	1:1T	+
5	1:16T	1:256	+
6	1:4T	1:256	+
7	1:16T	1:256	+
8	1:16T	1:256	−
9	1:4T	1:256	−
10	1:4T	1:16	−

* DT = Dye test titer.
† T = Thousand.

TABLE 4

Guinea pig enucleation at six months

Guinea Pig Number	DT* at 1–2 mo. Post-inoculation	DT at 4 mo.	Recovery of Organisms
1	1:16T†	1:4T	−
2	1:16T	1:4T	+
3	1:16T	1:4T	−
4	1:16T	1:4T	−
5	1:16T	1:1T	−
6	1:16T	1:1T	−
7	1:16T	1:1T	+
8	1:4T	1:1T	−
9	1:16T	1:1T	−
10	1:16T	1:16T	+

* DT = Dye Test Titer.
† T = Thousand.

TABLE 5

Guinea pig enucleation at eight months

Guinea Pig Number	DT* at 1–2 mo. Post-inoculation	DT at 4 mo.	DT at 8 mo.	Recovery of Organisms
1	1:16T†	1:256	1:4T	−
2	1:4T	1:256	1:4T	−
3	1:16T	1:256	1:1T	+
4	1:16T	1:256	1:256	−
5	1:16T	1:64	1:256	−
6	1:4T	1:256	1:256	+
7	1:4T	1:64	1:256	+

* DT = Dye Test Titer.
† T = Thousand.

TABLE 6

Guinea pig enucleation at 10 months

Guinea Pig Number	DT* at 1–2 mo. Post-inoculation	DT at 4 mo.	DT at 8 mo.	Recovery of Organisms
1	1:4T†	1:4T	1:4T	+
2	1:16T	1:16T	1:4T	+
3	1:16T	1:4T	1:1T	−
4	1:4T	1:4T	1:1T	+
5	1:16T	1:4T	1:1T	+
6	1:4T	1:1T	1:256	+
7	1:16T	1:1T	1:256	−
8	1:4T	1:4T	1:256	+
9	1:6T	1:4T	1:4T	+
10	1:4T	1:4T	1:4T	+

* DT = Dye Test Titer.
† T = Thousand.

A very low percentage of animals is lost as a result of the examinations and dissemination of the infection. Other observers will find this a satisfactory type of infection to produce from many standpoints.

It is interesting that the organisms can be inoculated into the relatively thick vitreous gel, and find their way to the chorioretinal tissues to produce an infection. Such a result seems to confirm previous observations of motility in this organism. It seems probable but not proved that the ocular infection is followed by dissemination of the organisms throughout the body and that this infection results in the observed serologic changes. We do not believe that the ocular inflammation alone would be sufficient to result in the serologic changes observed in the animals studied in these two experiments.

These investigations demonstrate satisfactorily that, following subsidence of the acute inflammation, organisms persist in the chorioretinal tissues for periods up to 10 months, and can be recovered in a high percentage of cases from the enucleated eyes. One animal was encountered in which no detectable chorioretinal inflammation occurred, yet organisms were recovered from the eye. It is possible that a small focus of inflammation occurred which was not observed with the ophthalmoscope. It is also interesting that organisms were recovered from the eye of one guinea pig at the time it had a titer of 1:16. This is comparable to what has been observed in probable acquired human infections.

Summary

1. After inoculation of approximately 5,000 live toxoplasmas into the vitreous of 60 guinea pig eyes, ophthalmoscopic evidence of chorioretinitis was present in 52 animals, 21 of which are under continued observation. The acute stage of the inflammation bore a marked resemblance to that observed in human ocular chorioretinitis.

2. All animals developed high dye test titers within a month after inoculation, indicating that local and systemic infections had occurred as the result of the eye injections.

3. Toxoplasmas were recovered from a significant percentage of the experimentally infected eyes of guinea pigs up to a period of 10 months after inoculation. The ability to recover organisms did not correlate with the severity of the initial infection, the height of the dye test titer, or the rapidity with which the titer dropped or changed.

1500 Fifth Avenue (22).

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Discussion

Dr. Ralph W. Ryan (Morgantown, West Virginia): I am pleased that research is actively continuing on the effects of chemotherapy on toxoplasmosis, which is one of the stated purposes of the study by Dr. Hogan and his colleagues. Evidence is accumulating to implicate the toxoplasma organism as one of the chief agents in the causation of chorioretinitis in humans.

The easy and rapid production in experimental animals of uniform ocular infections which resemble human toxoplasmic chorioretinitis and which would simulate the human disease in its reaction to therapy would be a notable achievement. Partial successes have been recorded. Frenkel by subcutaneous or intraperitoneal inoculation with toxoplasma organisms in golden hamsters produced

chorioretinal lesions apparently resembling chronic lesions in humans and containing both proliferative and encysted organisms. Hogan, in 1951, produced chorioretinal lesions in rabbits by intracarotid inoculation of toxoplasma organisms. Jacobs and others, by anterior chamber inoculation, produced an anterior type of uveitis in rabbits. More recently, Jacobs inoculated toxoplasma parasites directly into the retina of rabbits with production of chorioretinal lesions. In some of these investigations, chronicity of the lesion was sought by such measures as preliminary infection with a nonlethal strain of toxoplasma, followed by inoculation of such strains as the RH strain. Others made use of suppressive treatment with sulfa drugs after inoculation of virulent organisms.

It is interesting that all the guinea pigs in Dr. Hogan's Experiments I and II developed significant dye test titers within one month after inoculation in the vitreous. This suggests that the infection quickly reached other organs of the body so that a systemic infection resulted or that previous speculation concerning minimal antibody response from ocular lesions is not necessarily true for acute ocular infections. The preliminary copy of Dr. Hogan's paper failed to state whether proliferative forms or cysts were seen in the histopathologic preparations. Organisms, however, were found up to 10 months in a high percentage of cases, whereas he states that acute inflammation had subsided. The presence or absence of cysts may well be a factor of great importance in regard to treatment. There is considerable logic in the present assumption of some investigators that such drugs as sulfadiazine and pyrimethamine have little if any effect on the encysted organisms.

The study by Dr. Hogan and his associates adds to the accumulating evidence that toxoplasma parasites may persist in host tissues for long periods of time with or without clinical manifestation of disease. He also found no correlation between persistence of organisms and severity of initial infection, height of the dye test titer, or rapidity of titer changes.

I feel that this paper has value in describing a technique for easy production of ocular toxoplasmic infections in guinea pigs. Further study will show whether this animal preparation reacts to therapeutic agents in a manner similar to the toxoplasmic chorioretinitis occurring in humans. If this can be demonstrated, then we can more rapidly and safely test chemotherapeutic and immunologic measures to control the effects of this protozoan parasite. We are indebted to Dr. Hogan for the promise that this is only the beginning of a series of studies pointed toward the greater understanding and control of chorioretinitis due to the toxoplasma parasite.

Dr. Michael J. Hogan (closing): I thank Dr. Ryan very much for his kind discussion.

We attempted the production of experimental infections in a variety of animals, and most of them had the disadvantage cited by Dr. Ryan, namely, that the infection was so lethal for the animal that it was not possible to produce chronic ocular lesions. Other animals, such as the monkey, were so resistant that chorioretinitis could not even be produced by intraocular injection, which is remarkable. However, I think we now have a good experimental lesion which can be studied.

We attempted to duplicate Dr. Frenkel's work with golden hamsters and, in two series of experiments, were not able to produce the late ocular lesions which he described.

I failed to state that both proliferative forms and pseudocysts were found in the retinas of the experimental animals. In the later stages it was more common to find the pseudocysts.

OCULAR PENETRATION OF ANTIBIOTICS BY IONTOPHORESIS*

S. H. Witzel, M.D., Ingrid Z. Fielding, and Hugh L. Ormsby, M.D.
Toronto, Ontario

Many studies on the penetration of antibiotics into the aqueous humor have appeared in the literature in recent years. It was the purpose of the present study to review the ocular penetration of the commonly used antibiotics, and to compare the aqueous levels obtained by the usual methods of administration with those following iontophoresis.

Iontophoresis is not a new technique. Its usefulness is based on the fact that like charges repel one another, and that electrolytes administered by this method may reach higher tissue concentrations than by topical application. The method is especially adaptable to ophthalmic use because of the small area to be treated but has not been widely used because of the cumbersome equipment required, the slight discomfort experienced by the patient, and because it is more time consuming than other methods.

* From the Departments of Ophthalmology and Bacteriology, Faculty of Medicine, University of Toronto.

Materials and Methods

Adult rabbits were used throughout, and aqueous specimens were withdrawn under intravenous Nembutal anesthesia. The antibiotics were supplied by the manufacturers, and are referred to under the trade name of the company in each instance.

Following the removal of the aqueous with a No. 27 gauge needle on a two-cc. syringe, the antibiotic was assayed by a serial dilution method similar to that of Rammelkamp,[1] using the Hartley's broth as the nutrient medium. The inoculum consisted of a 1:10,000 dilution in Hartley's broth of a 24-hour culture at 37°C. The tubes were read after 24 hours, noting the presence or absence of gross turbidity. Staphylococcus aureus (peoria) was the test organism in all cases except for Polymyxin B, in which a strain of Ps. pyocyanea was used, and Chloromycetin, for which a strain of Pasteurella cuniculicida was found to be suitable.

All drugs were administered to groups of rabbits by subconjunctival injection, corneal bath (five minutes), and by iontophoresis. Those drugs which were suitable for parenteral administration were injected intravenously or into the muscles. Iontophoresis was carried out initially with the Selinger[2] plastic eyecup electrode, but it was later found that by burying the electrode in a cotton pad soaked with the drug solution, and placed over the loosely closed lids, identical results could be obtained. This method simplified the technique of administration and was used thereafter.

Results

1. Tetracyclines

Aureomycin hydrochloride was administered to adult rabbits in the following ways: intravenously in a dosage of 50 mg. per kg., subconjunctivally in a dosage of 2.5 mg. in 0.5 cc. of distilled water, as a corneal bath (20 mg. per cc.), and, using the same solution, by both anode and cathode iontophoresis for five minutes with a current of 2.5 ma.

Terramycin hydrochloride and achromycin hydrochloride were administered in the same dosage using the same methods. In all cases blood and aqueous specimens were withdrawn two hours following administration.

These methods resulted in poor ocular penetration with both aureomycin and terramycin, falling below 0.05 μg. per cc. by all methods with aureomycin, and below 0.12 μg. per cc. with terramycin.

Achromycin, when given by the intravenous route, reached aqueous levels between 0.5 and 3.0 percent of blood levels. These levels were lower than those which Abraham and Burnett[3] obtained, namely 10 percent—and the highest blood levels obtained were 30 μg. per cc. Aqueous concentrations following all other methods of administration did not exceed 0.12 μg. per cc.

With all the tetracyclines, topical and subconjunctival routes of administration resulted in a severe local reaction. Leopold and co-workers[4, 5] have shown that by exceeding this dosage and by abrading the cornea, low aqueous levels with the tetracyclines could be obtained, and these persisted for as long as two hours.

2. Chloramphenicol

Chloramphenicol base was used throughout, and was administered by the following routes: intravenously in a dosage of 50 mg. per kg., intramuscularly in the same dosage, subconjunctivally in a dosage of 2.5 mg., and by corneal bath in a concentration of 2.5 mg. per cc. for five minutes. Using the same solution, the drug was administered by iontophoresis, using the anode in one group of rabbits and the cathode in another. A current of 2.0 ma. was applied for five minutes in each case.

In all eyes, aqueous was withdrawn two hours after administration of the drug. The results of these experiments are seen in Table 1. Moderate levels in the aqueous

TABLE 1

AQUEOUS CONCENTRATION OF CHLORAMPHENICOL BASE TWO HOURS AFTER ADMINISTRATION

Method of Administration	Plasma Concentration (μg./cc.)	Aqueous Concentration (μg./cc.)
Intravenous 50 mg./kg.	8.0	2.2
Intramuscular 50 mg./kg.	4.0	1.5
Subconjunctival 0.5 cc. 2.5 mg./cc.		2.6
Corneal bath, 5 min. 2.5 mg./cc.		1.5
Anode iontophoresis 5 min. 2.0 ma. 2.5 mg./cc.		1.3
Cathode iontophoresis 5 min., 2.0 ma. 2.5 mg./cc.		1.3

were obtained by all routes of administration. Thus our results agreed fairly closely with those of Leopold and co-workers,[6] and with those of Abraham and Burnett.[3] Iontophoresis did not give higher levels than corneal baths, due to the fact that the drug was not ionized in solution.

3. The medium-spectrum group of antibiotics

Both Magnamycin and Ilotycin are bacteriacidal to gram-positive and gram-negative cocci, but have little effect on the gram-negative bacilli. Ilotycin is readily absorbed from the gastrointestinal tract, whereas Magnamycin is not.

Since ocular penetration of Magnamycin has already been reported from this laboratory, and has been shown to reach therapeutic concentrations by all methods of administration, only a few experiments were done in the present study. Two eyes were subjected to anode iontophoresis for five minutes at 2.0 ma., using an aqueous solution containing 20 mg. per cc. of Magnamycin sulfate. This resulted in an aqueous level of 4.0 μg. per cc. in each eye at the end of two hours. A corneal bath for five minutes, using the same solution, was applied to two eyes. Two-hour aqueous levels were approximately 1.0 μg. per cc.

Ilotycin glucoheptonate was administered intravenously in a dose of 50 mg. per kg., subconjunctivally (0.5 cc. of an aqueous solution containing 20 mg. per cc.), by corneal bath (20 mg. per cc. for five minutes), and by anode iontophoresis for five minutes with a current of 2.0 ma.

The results of these experiments will be seen in Table 2. Therapeutic levels were obtained in the aqueous by all routes of administration. Iontophoresis gave somewhat higher levels than did the corneal bath, but did not equal those obtained after subconjunctival injections.

4. Penicillin

Potassium penicillin G was administered intravenously in a dosage of 10,000 units per kg., by subconjunctival injection (0.5 cc. of a solution containing 50,000 units per cc.), by corneal bath (50,000 units per cc. for five minutes), and, using the same solution, by both anode and cathode iontophoresis with a current of 2.0 ma. for five minutes. The results of these experiments are seen in Table 3.

The low levels of penicillin in the aqueous following intravenous injection agree with the previous reports of von Sallmann,[7] and are due in part to the fact that penicillin becomes protein-bound. The high levels following subconjunctival injection are 10 times those reported by von Sallmann, but the dosage employed in our experiments was 10 times higher than his. Cathode iontophoresis

TABLE 2

AQUEOUS CONCENTRATION OF ILOTYCIN GLUCOHEPTONATE TWO HOURS AFTER ADMINISTRATION

Method of Administration	Plasma Concentration (μg./cc.)	Aqueous Concentration (μg./cc.)
Intravenous 50 mg./kg.	3.7	1.3
Subconjunctival, 0.5 cc. 20 mg./cc		25
Corneal bath, 5 min. 20 mg./cc.		0.5
Anode iontophoresis, 5 min., 2.0 ma. 20 mg./cc.		2.6

TABLE 3

AQUEOUS CONCENTRATION OF POTASSIUM PENICILLIN G TWO HOURS AFTER ADMINISTRATION

Method of Administration	Plasma Concentration (units/cc. 2 hr.)	Aqueous Concentration (units/cc. 2 hr.)
Intravenous 10,000 units/kg.	1.3	0.1
Subconjunctival, 0.5 cc. 50,000 units/cc.		26
Corneal bath, 5 min., 50,000 units/cc.		2.5
Cathode iontophoresis 5 min., 2.0 ma. 50,000 units/cc.		20
Anode iontophoresis 5 min., 2.0 ma. 50,000 units/cc.		2.5

resulted in levels 15 times those obtained by anode iontophoresis, and the latter were the same as those following corneal bath.

5. STREPTOMYCIN-CALCIUM-CHLORIDE COMPLEX AND DIHYDROSTREPTOMYCIN SULFATE

These antibiotics were administered intramuscularly in dosages of 20 and 100 mg. per kg., subconjunctivally, by corneal bath for five minutes in unabraded eyes, by anode iontophoresis for five minutes, and by cathode iontophoresis in both normal and abraded eyes. The solutions used throughout contained 20 mg. of antibiotic per cc.

While measurable levels in the aqueous were obtained by all methods, the highest concentrations were obtained by subconjunctival injection, and by anode iontophoresis. The results are presented in Tables 4 and 5.

6. NEOMYCIN SULFATE

Neomycin sulfate, Polymyxin B, and Bacitracin were studied only by local administration, since they are rarely given to patients by systemic routes.

Neomycin sulfate was administered by corneal bath containing 20 mg. per cc. for five minutes, by subconjunctival injection (1.2 mg. in 0.5 cc. of distilled water), and

TABLE 4

AQUEOUS CONCENTRATION OF STREPTOMYCIN-CALCIUM-CHLORIDE COMPLEX TWO HOURS AFTER ADMINISTRATION

Method of Administration	Aqueous Concentration μg./cc.
Subconjunctival, 0.5 cc. 20 mg./cc.	1.6
Corneal bath, 5 min. 20 mg./cc.	0.6
Anode iontophoresis, 5 min. 2.0 ma. 20 mg./cc.	4.0
Cathode iontophoresis 5 min. 2.0 ma. 20 mg./cc.	0.6

TABLE 5

AQUEOUS CONCENTRATION OF DIHIDROSTREPTOMYCIN SULFATE, TWO HOURS AFTER ADMINISTRATION

Method of Administration	Plasma Concentration (μg./cc.)	Aqueous Concentration (μg./cc.)
Intramuscular 20 mg./kg.	10.6	1.1
Intramuscular 100 mg./kg.	27	1.4
Subconjunctival 0.5 cc. 20 mg./cc.		6.6
Corneal bath, 5 min. 20 mg./cc. (normal eyes)		0.3
Corneal bath, 5 min. 20 mg./cc. (4.5 mm. abrasion)		2.3
Anode iontophoresis 5 min., 2.0 ma. 2 mg./cc. (normal eyes)		10.0
Anode iontophoresis 5 min., 2.0 ma. 20 mg./cc. (4.5 mm. abrasion)		5.0
Cathode iontophoresis 5 min., 2.0 ma. 20 mg./cc. (normal eyes)		0.8

by anode iontophoresis with a solution containing 10 mg./cc., using a current of 2.0 ma. for five minutes. The results of these experiments are seen in Table 6.

While measurable aqueous concentrations were obtained by all methods, the highest levels followed anode iontophoresis.

7. POLYMYXIN B

Polymyxin B sulfate was administered in a dosage of 0.5 cc. of an aqueous solution containing 2,000 units per cc., by subconjunctival injection, corneal bath for five

TABLE 6

AQUEOUS CONCENTRATION OF NEOMYCIN SULFATE TWO HOURS AFTER ADMINISTRATION

Method of Administration	Aqueous Concentration μg./cc.
Subconjunctival, 0.5 cc. 2.5 mg./cc.	0.5
Corneal bath, 5 min. 20 mg./cc.	0.9
Anode iontophoresis 5 min., 2.0 ma. 10 mg./cc.	3.8

minutes, and by anode and cathode iontophoresis for five minutes with a current of 2.0 ma. By all methods of administration the drug failed to reach detectable concentrations (0.24 units per cc.) in the aqueous.

Polymyxin B sulfate was then administered by the same methods using a solution containing 100,000 units per cc. All eyes exhibited a severe inflammatory reaction which subsided in five days. No detectable Polymyxin was present in the aqueous after the corneal bath or cathode iontophoresis. An aqueous concentration of 0.24 units per cc. was present two hours after subconjunctival injection, and a level of 0.48 units per cc. was found two hours following anode iontophoresis.

8. BACITRACIN

Bacitracin was administered by the methods used with Polymyxin B, the solution containing 1,000 units per cc. No measurable aqueous levels (10 units per cc.) were obtained.

DISCUSSION

The results of these penetration studies with the antibiotics are similar to those previously reported in the ophthalmic literature. Our results differ in the following respects. Leopold and co-workers[5] and Abraham and Burnett[3] found slightly higher penetration of achromycin, and with Chloromycetin[3,6] they have found aqueous concentrations in general about half those of the blood. In our study, the aqueous levels were approximately one third of the blood level. Ainslie and Smith[8] reported significant aqueous levels of Polymyxin E following subconjunctival injection, using up to 10 times the dosage employed in our study. They do not feel that the inflammatory reaction produced by such doses is sufficiently severe to preclude the clinical use of this particular drug. Our experimental work with Polymyxin B, however, suggests that such dosage with this antibiotic subconjunctivally would result in local reactions of extreme severity.

From the tables it will be seen that iontophoresis has no advantage over other methods with the antibiotics which do not ionize in solution (the tetracyclines, Chloromycetin and bacitracin) or which do not penetrate by other methods (Polymyxin). These results will be seen in Table 7.

With streptomycin, dihidrostreptomycin, and neomycin, anode iontophoresis resulted in the highest aqueous levels obtained in the dosages employed in this study. With penicillin, cathode iontophoresis proved superior to

TABLE 7

EFFECT OF IONOTOPHORESIS ON AQUEOUS LEVELS OF NONELECTROLYTES AND POLYMYXIN B

Antibiotic	Method of Administration				
	Intramuscular	Intravenous	Subconjunctival	Topical	Iontophoresis
Chloramphenicol	1.5 μg.	2.2 μg.	2.6 μg.	1.5 μg.	1.3 μg. (anode & cathode)
Aureomycin		<0.05 μg.	<0.05 μg.	<0.05 μg.	<0.05 μg. (anode & cathode)
Terramycin		<0.12 μg.	<0.12 μg.	<0.12 μg.	<0.12 μg. (anode & cathode)
Achromycin		0.5–3% of blood level	<0.12 μg.	<0.12 μg.	<0.12 μg. (anode & cathode)
Polymyxin B			0.24 units	<0.24 units	0.48 units (anode & cathode)
Bacitracin			<10 units	<10 units	<10 units (anode & cathode)

TABLE 8

COMPARATIVE AQUEOUS LEVELS OF ANTIBIOTICS TWO HOURS AFTER ADMINISTRATION

Antibiotic	Method of Administration				
	Intramuscular	Intravenous	Subconjunctival	Topical	Iontophoresis
Streptomycin			1.6 μg.	0.6 μg.	4.0 μg. (anode)
Dihidrostreptomycin	1.1 μg.		6.6 μg.	0.3 μg.	10 μg. (anode)
Neomycin			0.5 μg.	0.9 μg.	3.8 μg. (anode)
Penicillin		0.1 units	26 units	1.3 units	20 units (cathode)
Magnamycin				0.5 μg.	4.0 μg. (anode)
Ilotycin		1.3 μg.	25 μg.	0.5 μg.	2.6 μg. (anode)

both intravenous administration and corneal bath, and produced comparable levels to those obtained by subconjunctival injection. With Magnamycin and Ilotycin, anode iontophoresis was found more effective than corneal bath, but with the latter drug iontophoresis produced levels lower than those obtained by subconjunctival injection (table 8).

When using iontophoresis, the pole applied to the eye is of the utmost importance. When the active principle of the drug is present in the cation, iontophoresis should be from the anode. Reversal of the polarity results in levels similar to those obtained by corneal bath, as shown in Tables 3, 4, and 5. Whereas it is evident that abrasion of the cornea prior to corneal bath increases ocular penetration, it has no value during iontophoresis.

SUMMARY

The ocular penetration of 12 antibiotics into the aqueous of rabbits following iontophoresis has been compared to that by other methods of administration.

Identical results were obtained when the eye electrode was buried in cotton placed over the eye and soaked with the test solution with those obtained by means of the plastic eyecup electrode.

Iontophoresis resulted in the highest aqueous levels with streptomycin, dihydrostreptomycin, and neomycin. High levels, equal to other methods, were obtained by iontophoresis with penicillin, Magnamycin, and Ilotycin. This method was of no value with Polymyxin B, Bacitracin, or the tetracyclines.

University of Toronto.

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Discussion

Dr. Irving H. Leopold (Philadelphia): This paper by Dr. Witzel and his associates has practical value. Actually, it reviews the various methods which have been used in the past by other investigators and derives the same conclusion with minor exceptions.

As ophthalmologists we have to be concerned with the way in which antibiotics penetrate from the blood stream into the eye, and how they penetrate when locally applied. We cannot be satisfied with dosage schedules which have been worked out for other parts of the body. Many of the antibiotics that ophthalmologists would like to use do not penetrate well from the blood stream into the eye, and therefore must resort to such measures as cotton packs, corneal baths, subconjunctival injections, and iontophoresis.

Dr. Witzel has called our attention again to the use of iontophoresis. He also has pointed out the necessity of realizing that not all drugs will penetrate well when iontophoresis is used. Particular attention should be paid to the fact that terramycin, aureomycin, and tetracycline do not penetrate well when used by iontophoresis, and these are the very drugs which do not penetrate well when administered systemically, intravenously, orally, or intramuscularly. It would be helpful to have an innocuous method to improve the penetration of these agents. Iontophoresis does not serve this purpose.

Iontophoresis, although it may be valuable, is not widely used for several reasons. It requires a special apparatus. In spite of the use of a local anesthetic, iontophoresis causes some discomfort to the patient when the current is turned on and off. In addition, there has been some evidence, as presented by Dr. von Sallmann, that certain types of iontophoresis, namely, the anode variety, when the positive pole is placed on the eye, tends to produce more corneal damage than when the negative pole is placed on the eye.

There is some question as to how iontophoresis improves penetration of drugs through the cornea. Dr. Witzel and his co-workers do not feel that it is by virtue of denuding the epithelium but by alteration of the epithelial barrier in some other manner.

This was suggested by Dyson in the 1940's He showed that if you employ iontophoresis with salt solution prior to the application of the antibiotic to be studied, this in itself would increase the penetration of the antibiotic without producing epithelial damage.

Dr. Witzel and his co-workers have done us a service by reviewing much of the data available on antibiotics and confirming the bulk of it through their own investigations.

EXPERIMENTAL STUDY OF MODIFICATIONS OF THE SCLERAL FOLD OPERATION*

William G. Everett, M.D.
Pittsburgh, Pennsylvania

A new retinal detachment operation for shortening the sclera, the scleral fold, was introduced by me over one year ago.[1]

The application of this operation has achieved increasingly wide acceptance. Clinical experience and animal studies employing this scleral shortening technique have suggested several modifications of this procedure.

An experimental comparison in rabbits of the scleral fold, penetrating scleral resection, lamellar-scleral resection, and lamellar-scleral resection with infolded polyethylene tubing, revealed certain advantages and disadvantages of each of these scleral shortening operations.[2]

The lamellar-scleral procedures, with and without polyethylene tubing, were relatively atraumatic but tended to be less permanent. This was especially true of the technique utilizing polyethylene tubing, for the tubing was consistently extruded from the scleral buckle in several weeks. The penetrating scleral resection, although most traumatic to the choroid and retina, showed excellent scleral healing. The scleral fold operation was somewhat traumatic to the choroid because of the separation of the choroid and

* From the Department of Ophthalmology, University of Pittsburgh, School of Medicine, and the Eye and Ear Hospital. This study was supported in full by the Ophthalmic Foundation of Pittsburgh.

sclera by spatulization, but was safer and created less reaction than the penetrating scleral resection.

This study was in agreement with Della-porta,[3] who noted that penetrating scleral resections heal predominantly from the suprachoroid and to a lesser extent from episcleral elements. Healing of the lamellar-scleral types of operations can only take place from the episcleral tissues. The scleral fold operation is limited to healing from the suprachoroid, except for minor migration of episcleral cells by way of the suture tracts. Microscopic study of the wound in a penetrating scleral resection reveals the advantage of healing when both episclera and suprachoroid response are possible.

These animal experiments[2] suggested modifications of the scleral fold operation which would most nearly satisfy the criteria for safe and effective scleral shortening in retinal detachment.

The first modification of the scleral fold operation suggested was the abandonment of separation of the sclera and choroid by a spatula, thus hoping to avoid excess trauma to the choroid and possible hemorrhage. Microscopic sections of these previous experimental operations in rabbits had shown a normal scleral fold free of any choroid in areas that the spatula had not reached.

A second modification was suggested by the excellent healing of the penetrating scleral resection. It seemed logical that excision of a well sutured scleral fold, distal to the sutures, would allow elements of the episclera to aid more actively the suprachoroid in scleral healing, as is seen in penetrating scleral resections.

This study was conducted in an effort to make a comparative evaluation of the healing of excised and unexcised scleral fold operations in dog eyes. All the operations which comprise this study were performed without prior separation of the sclera and choroid by spatulization.

Experimental procedure

Scleral-fold operations were performed on both eyes of 10 dogs, a total of 20 eyes.

Under intravenous Nembutal anesthesia the temporal aspect of the globe was exposed by a conjunctival incision and reflection of the lateral rectus muscle. Decompression of the globe was accomplished by a paracentesis of the anterior chamber. With the globe thus softened, the sclera was tented at the equator by picking up the superficial scleral fibers with a needle held in a needle holder. A small bite of the tented sclera was then grasped with a forceps adjacent to the needle. The needle was released, and a second forceps was alternately used to grasp a larger amount of sclera. When about 2.0 mm. of sclera was caught up in a neat fold, the forceps were maintained by an assistant while a mattress suture of 4-0 black silk was placed in the base of the fold. A muscle hook was used to depress the globe in front of the fold as the needle was being passed through the base of the fold. This prevented the needle from penetrating the choroid if it were inadvertently passed too deep. The fold was extended on either side of the first suture, using the two forceps and applying a mattress suture about every 1.5 mm. apart until the fold was 15 mm. long.

The fold of sclera was excised distal to the sutures in one eye of each dog. The scleral fold in the fellow eye was left intact.

Two dogs were killed immediately postoperatively. Two each of the remaining eight dogs were killed at four days, 11 days, 21 days, and 45 days. There were four eyes for study in each of the five periods, two with excised scleral folds and two with intact scleral folds.

At the completion of each study period, the globes were enucleated and mounted in celloidin. Serial sections through the operated area were prepared and stained with hematoxylin-eosin and with Masson's stain for microscopic study.

Results of microscopic study

A. Immediate postoperative period (figs. 1 and 2)

The immediate postoperative results in unexcised and excised scleral-fold operations are essentially the same, except for the excised sclera in half the eyes.

Little scleral change is seen. This might be expected in view of the relatively inert nature of the sclera. In those operations in which the outer portion of the fold was excised, a well-delineated full-thickness strip of sclera can be seen to have been removed. In most of the photomicrographs, one can see an extensive venous plexus of the anterior one-third of the sclera, which often gave rise to annoying hemorrhage during operation.

The choroid remains free from the folded sclera other than conforming to the apex of the inner base of the fold. The lamina fusca is heavily pigmented in dogs and remains adherent to the coapted scleral surfaces. After excision of the scleral fold, examination of the removed strip reveals a few adherent fibers of the lamina fusca.

The choroid is congested and hyperemic in most cases. Occasionally hemorrhage of the choroid occurs at enucleation. When this happens the hemorrhage is fresh and usually massive, differentiating it from choroidal hemorrhage due to operative trauma.

All cases show a retinal fold compensating for the reduced ocular volume. This fold does not include the pigment epithelial layer. The fold of the retina is in the general area of the operation but not always adjacent to the scleral fold.

B. Four-day postoperative period (figs. 3 and 4)

The episcleral tissues show a subsiding acute inflammatory reaction in the operated area.

A similar reaction is seen along the suture tracts; otherwise there is little change in the sclera.

The suprachoroidal tissue shows some disorganization and migration of pigment cells in addition to an inflammatory response. A few fibroblasts are seen in this area.

Choroidal congestion is still present. In some instances this choroidal hemorrhage has extended to the subretinal space. Aside from folds, no retinal changes are noted.

C. Eleven-day postoperative period (figs. 5 and 6)

The episclera shows mainly chronic inflammatory cells at this time. Early scleral healing can be seen best at this time with Masson's stain which more clearly differentiates newly formed fibrous tissue. In both the excised and unexcised folds a fibrous bridge joining the sclera in the suprachoroidal area can be noted. In the unexcised folds there is a tendency toward gaping of the fold, with proliferation of fibrous tissue and a chronic inflammatory reaction within the fold. There is an attempt of the episcleral tissue to bridge the sclera where the sutures have begun to cut through the unexcised fold. This cutting of the sclera by the sutures in the unexcised folds gives the appearance of an attempt of the sutures to excise the distal portion of the fold, and seems to create a more marked inflammatory response of the episclera.

The excised folds show less inflammatory reaction of the episclera and less tendency to gaping of the scleral wound. However, by this time there is a uniform reduction of the amount of out-turning of the scleral lips. Healing at this period closely resembles that of a penetrating scleral resection, except there is little chorioretinitis in the scleral fold.

The choroidal and retinal reaction is subsiding, and is about the same in both types of operations. Retinal folds and some choroidal hyperemia persist.

Fig. 1 (Everett). Intact scleral fold immediately postoperative.

Fig. 2 (Everett). Excised scleral fold immediately postoperative.

Fig. 3 (Everett). Intact scleral fold four days postoperative.

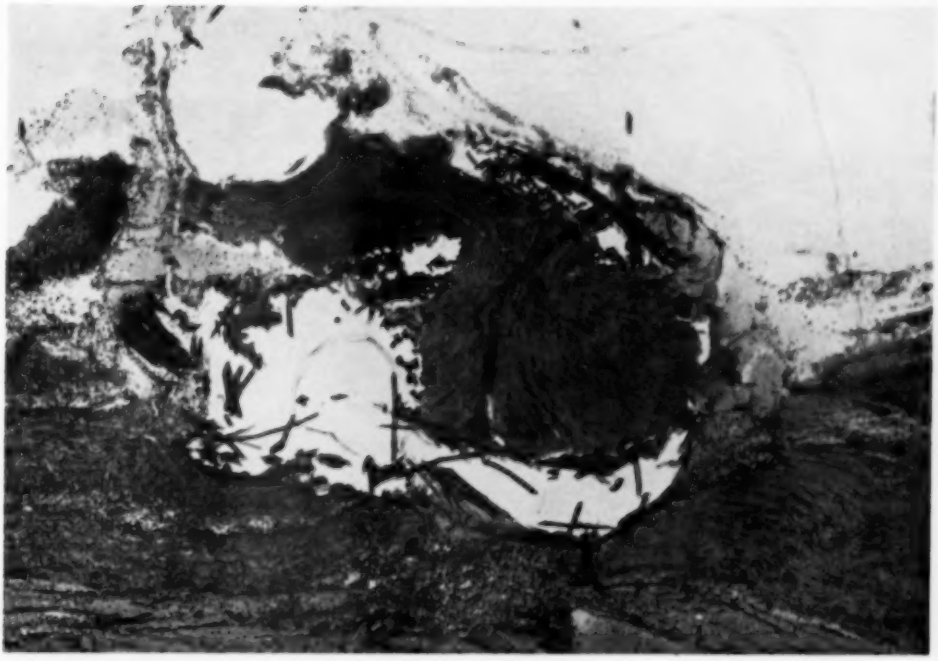

Fig. 4 (Everett). Excised scleral fold four days postoperative.

D. TWENTY-ONE DAY POSTOPERATIVE PERIOD (figs. 7 and 8)

There is a definite reduction of the scleral fold in both types of operation. The unexcised fold shows a ridge of out-turned sclera about equivalent to the amount of sclera removed in the excised scleral fold procedures. The effective scleral shortening appears to be about equal at this period.

The episcleral inflammatory response is

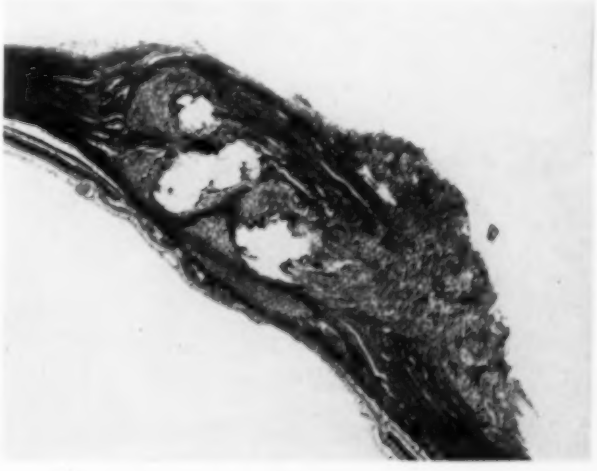

Fig. 5 (Everett). Intact scleral fold 11 days postoperative.

Fig. 6 (Everett). Excised scleral fold 11 days postoperative.

Fig. 7 (Everett). Intact scleral fold 21 days postoperative.

Fig. 8 (Everett). Excised scleral fold 21 days postoperative.

more consolidated and is characterized by giant cells in both procedures. The fibrous bridge at the base of the unexcised fold fills the apex of the fold and contains pigment granules. A fibrous bridge is also present over the fold.

It is difficult to identify the excision line in the sclera of the excised fold. More uniform continuity of the sclera with a less marked inflammatory response is noted.

The choroid and retina show little change.

E. Forty-five day postoperative period (figs. 9 and 10)

The operated site is marked by large giant cell aggregations about the suture remnants in the episclera. Bands of fibrous tissue course through these aggregations. In the operated area there is a definite and unbroken continuity of the sclera. At this period there is little if any microscopic difference between the unexcised and excised scleral folds.

The choroidal congestion is no longer present.

The globe appears to have resumed its normal dimensions and the retina no longer shows compensatory folds.

Conclusions of microscopic study

This study shows convincingly the practicality of performing scleral fold operations without prior separation of the choroid and sclera. The degree of choroidal and retinal trauma sustained during scleral shortening by a fold operation is negligible as long as the globe is adequately decompressed preoperatively.

In comparing unexcised and excised scleral fold operations, there appears to be somewhat less inflammatory reaction following the excised scleral-fold operation. The healing in an excised scleral-fold operation more closely resembles the healing of a conventional penetrating scleral resection, without the same marked evidence of chorioretinal trauma.

This study did not demonstrate any appreciable difference in the permanency of shortening in either the unexcised or excised scleral-fold operations. Volumetric studies were not undertaken.

Discussion

Choroidal hemmorhage and congestion of the choroid are a prominent feature of this study. Similar choroidal disturbance was uniformly absent in scleroplasties studied in rabbits.[2] Capper and Leopold,[4] in studying choroidal detachment in rabbits, noted the failure of the choroid to detach following trauma to the sclera unless accompanied by prolonged hypotony obtained by a fistulization of the anterior chamber. Swan feels that choroidal detachment can be observed in a high percentage of scleroplasties in humans,

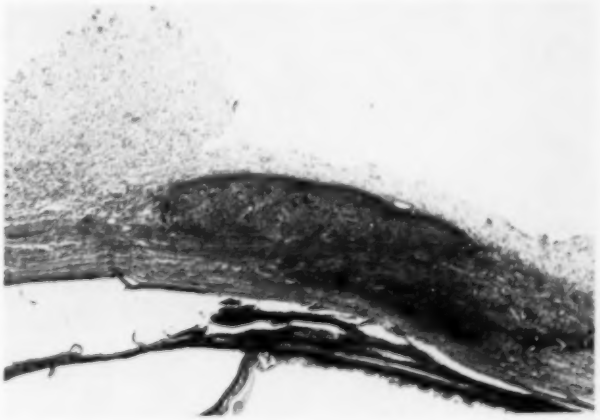

Fig. 9 (Everett). Intact scleral fold 45 days postoperative.

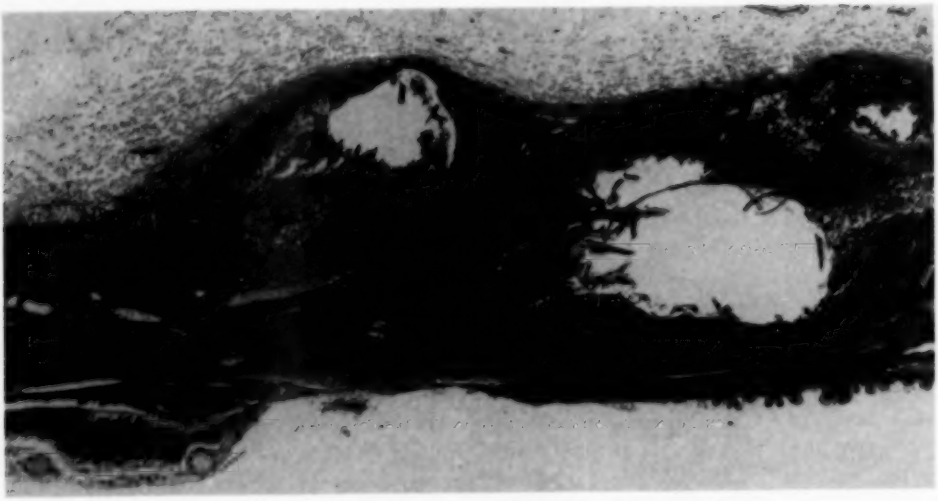

Fig. 10 (Everett). Excised scleral fold 45 days postoperative.

immediately after operation. Swan, Christiensen, and Weisel[5] substantiated this observation by experimental choroidal detachment in dogs following shrinkage of the sclera by diathermy. However, dog choroid detaches very easily and animal choroids in general vary greatly in their response to hypotony or trauma. More study of the relationship of choroidal and retinal detachment in humans is necessary.

The shortening effect of the scleral fold operation in this experiment is well demonstrated by the compensatory retinal folds seen shortly after operation. Clinically, postoperative folds of retina will be dependent on the status of the detached retina. If there has been shrinkage of the retina after detachment, a scleral shortening operation may not create retinal folds.

The over-all shortening effect of a scleral fold operation is more generalized at the wound site and not associated with a prominent ridge of choroid. For this reason it is not essential that the operation be directly over the retinal hole. It is essential that the choroid and retina be brought in contact at the site of the retinal tear. This may be achieved by altering the amount and location of the sclera included in the fold. As much as seven or eight mm. of sclera can be drawn into a fold if care is used to take small progressive bites of sclera.

In lamellar-scleral operations with polyethylene tubing a prominent indented ridge of choroid is created at the operative site. This ridge of choroid must be placed so that it contacts the retina posterior to the retinal tear. In equatorial or posterior retinal holes, operation by this technique may be mechanically difficult.

In all types of scleral shortening operations in animal eyes, permanency of the amount of shortening seems questionable after microscopic study. Volumetric studies of dog eyes have been done by Scheie and Bourne,[6] who felt they demonstrated less constant reduction of ocular volume following penetrating scleral resections than that obtained by shrinkage of the sclera by surface diathermy. The nature of animal vitreous is a most important factor in experimental scleral shortening. The healthy, firm vitreous of animals offers great resistance to reduction of ocular volume and diminishes the permanency of experimental scleral shortening. Clinical retinal detachments in humans are usually associated with an abnormal or fluid vitreous which is less re-

sistant to scleral shortening procedures.

Individual scleroplasties in humans may show marked alteration in refraction. However, studies of refractive changes in a series of scleral shortening operations have not shown consistently permanent alteration in the antero-posterior length of the eye. Clinical experience suggests, if scleral shortening is not entirely permanent in successful retinal detachment operations, that the return of the eye to increased dimensions must be very gradual and allow compensatory stretching of the retina.

Summary

A previous experimental study of the basic scleroplasty procedures used in retinal detachment therapy suggested two modifications of the scleral-fold operation. It was thought that with these changes the scleral-fold operation would more nearly include the desirable features of all scleral shortening procedures. The suggested modifications of the scleral-fold operation were: abandonment of separation of sclera and choroid preoperatively, and excision of the folded sclera at the completion of a scleral fold.

This experiment was designed to evaluate these modifications in dog eyes.

The results of this study showed spatulization of the choroid and sclera to be unnecessary in scleral folding.

Excising the top of the scleral fold demonstrated better scleral healing with less inflammatory reaction. Permanency of shortening could not be evaluated microscopically in either the unexcised or excised scleral fold; volumetric determinations were not a part of this study.

Some of the factors of importance in evaluating experimental scleral shortening are discussed.

401 Jenkins Building (22).

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Discussion

Dr. Bayard Colyear, Jr. (San Francisco): I am here this afternoon in the position of a pinch-hitter, so I hope that if my comments are somewhat in disorder you will excuse them.

I want to thank Dr. Everett for having continued his studies of the reactions of scleral shortenings in dog eyes and rabbit eyes, and I would like to take this opportunity to urge those of you present to remember that our eye-banks still can accept a lot of specimens from us; so please remember on your detachment cases to try and make an effort to have donor forms signed.

In recent years much of the detachment surgery literature has been in regard to scleral resection. Still, the majority of retinal detachments can be cured (if they are found early) by diathermy detachment procedures, and I believe we should regard scleral shortening operations as ancillary procedures in the treatment of retinal detachments.

Our feeling about the action of a scleral resection is that it provides slack to the vitreous, allowing the vitreous to loosen its pull on the retina, and allowing the retina to reappose itself to the choroid. Scleral shortening is also applicable in cases of retinal shortage, in which case the area of the choroidal and scleral bed is made to conform with the decreased area of the retina.

I was not quite clear when Dr. Everett said that a lamellar resection had to be central to the tear. Most of our resections are not central to the tear, and very often they may be 180 degrees opposite the tear. We use a scleral resection to slack off on the vitreous pull on the retina.

The permanency of a resection of any type, as Dr. Everett pointed out, is very difficult to determine. Clinically we have had the unwelcome opportunity of examining a number of our resections a year and two years afterward when the retina came off again. The scleral wound certainly looked well healed, both in total penetrating and lamellar scleral

resections. Attempts to evaluate clinically the change in ocular size by refractive error are a little dubious because many of the changes are those that affect the curvature of the anterior section of the globe, which has a considerable refractive impact.

I do agree with Dr. Everett that probably, even if the volume of the globe returns to its original status, the retina has had enough time to relax and get back to its original area so that perhaps permanency by using a polyethylene tube is not necessary, and one can with these other resections allow the retina time enough to get back.

I think Dr. Everett's operation has one very strong point in its favor. Often when a lamellar scleral resection is outlined it is difficult to determine exactly how much should be performed, and one hopes that the last two sutures pull up well and that the globe is not too firm making it too difficult to close the wound well. If Dr. Everett's procedure is followed, one can stop when the globe becomes firm. If fundus examination reveals that more subretinal drainage is necessary, this can be performed and the scleral outfolding continued. If one looks into the fundus and the retina is apparently well in place everywhere, then one is in a position to stop the scleral resection immediately without having uncovered more choroid than necessary. It is rather exasperating to have eight mm. of lamella still present to be closed when the eye is firm and there are no immediate prospects for further drainage. In this latter situation, one must try for further subretinal drainage to allow enough softening of the globe or, if the retina is flat, resort to paracentesis. Softening of the eye from paracentesis, of course, is removing fluid from in front of the retina, which is not very desirable.

Dr. A. Edward Maumenee (Baltimore): This is a very interesting technique that Dr. Everett has advocated for doing scleral resections. There are a couple of questions that I would like to ask. First, is it possible to remove a large area of sclera, four- or five-mm. wide, without including the choroid in the scleral resection? In the slide presented, showing the scleral buckle procedure, only a small piece of sclera was excised. It appears that if a wide resection was done the choroid might buckle along with the sclera.

Next, there are instances where the retina is detached and a number of vitreous strands are present that pull the retina away from the choroid. In such cases, it is sometimes of value to push the choroid into the eye so that it will come into apposition with the retina. Schepens has advocated the use of polyethylene tubes in such patients. Does the author think that his technique would be as effective in such patients?

Finally, the author has emphasized the small amount of reaction that occurs in the area of the scleral resection using his technique. Clinically, we have found that it is important to produce a choroiditis in the area of a scleral resection so that the retina would become adherent to the choroid in this area. Thus, diathermy is applied to the choroid in the area of resection. I wonder if the essayist feels that such reaction is contraindicated in a scleral resection.

Dr. Charles L. Schepens (Boston): Dr. Everett is to be congratulated for his careful microscopic study and clear exposition of his modifications to the scleral fold operation. His observation that the scleral fold operation, like other types of scleral resection, does not reduce permanently the volume of the globe is of particular interest.

In his discussion, Dr. Everett speculates as to the value of his operation for retinal detachment in human eyes. I think that the applicability of his procedure to humans will be found to have unforeseen limitations; for instance, it will probably be impossible to perform this operation in cases where the sclera is either very thin or very edematous. Dr. Everett also indicates that his procedure may be used advantageously in place of a scleral buckling with implantation of polyethylene tube. This statement will require the support of further clinical investigation.

Dr. William G. Everett (closing): I will try to answer the questions briefly.

First of all, I thank Dr. Colyear and Dr. Maumenee for being kind enough to discuss my paper extemporaneously.

Dr. Colyear, in my haste I forgot to qualify that with the polyethylene operation the tubing has to be placed posterior to the fold.

In answer to Dr. Maumenee, in terms of size, we have taken up as much as eight mm. of sclera in one of these folds, but it must be done very carefully, taking very small bites as you enlarge the fold not including sclera. This was an elliptical strip, 25-mm. long and 8.0-mm. wide. We have also circled the globe with almost 360 degrees of fold, so you can alter and vary this at will, almost, if you use care not to pinch the choroid up.

As far as vitreous retraction is concerned, my own experience has been extremely disappointing, and those with whom I have spoken have felt the same way. In massive vitreous retraction I have tried all procedures I have heard of or known of, and I have been uniformly unsuccessful. If it is a vitreous retraction band, I think you can place a scleral fold opposite the line of traction, and get the same degree of shortening as with any of the other procedures, if you so desire, but with massive vitreous retraction I think the answer lies in the vitreous and not the sclera.

As far as the chorioretinal reaction is concerned, I agree with you and we do use diathermy around the operated area. I am referring here particularly to the penetrating scleral resection. This is what started me in this work. When I first started to learn to do resection work, I learned the penetrating resection, and was so appalled at the amount of vitreous I saw, and the amount of hemorrhage, that I felt I would either give up scleral resection surgery or do something different. Now I do the scleral fold operation.

Dr. Schepens' predicted limitations of this operation have not been borne out by our clinical experience in over 75 cases treated by the scleral fold operation.

THE ARGYLL ROBERTSON PUPILLARY SYNDROME*

Mechanism and Localization

Otto Lowenstein, M.D.
New York

The question of the mechanism by which the Argyll Robertson syndrome is produced belongs to the unsolved problems which were passed on from the neurologists and ophthalmologists of the 19th century to those of the 20th century. Although this syndrome has been known clinically for almost 90 years and there is a vast literature dealing with it, the physiologic and anatomic basis is still controversial. I do not expect in this paper to give the final solution, but I will try to separate the grain from the chaff and will discuss the results of some new experiments which may possibly bring us somewhat closer to the solution.

According to the classical description, the Argyll Robertson[1, 2] syndrome is characterized by three essential features:

1. Absence or diminution of the pupillary reflex to light.
2. Active or supposedly hyperactive pupillary contraction to near vision.
3. Miosis.

I am going to deal with each of these features. The combination of absence or diminution of pupillary contraction to light with active contraction to near vision will be referred to as the "dissociation phenomenon."

I. Methods and Material

Methods and material are the same as those outlined in a previous paper.[3]

Pupillary diameter and reactions were recorded by pupillography as previously described and illustrated.

The general statements on pupillary innervation contained in this paper are based on the entire material collected in this laboratory. It includes approximately 2,900 clinical cases with pupillary pathologic processes of all kinds; among them are clinical pupillograms obtained from more than 130 patients with the Argyll Robertson syndrome in the classical sense. In addition, about 100 clinical cases were included in which the Argyll Robertson syndrome was not fully developed but was considered to be in an initial stage.

In some of these initial cases, the pupillary reaction to light was not completely abolished but was reduced while the reaction to near vision was preserved, creating a relative but not an absolute dissociation phenomenon. In other initial cases complete dissociation was present, but the pupillary diameter was at or near the lower physiologic limit and measured about 5.0 mm. after adaptation to darkness ([1] table 1, page 357); they were therefore less miotic than those in the group of the classical Argyll Robertson syndrome.

Animal experiments were done on monkeys and cats; the experimental animals were pupillographically examined many times before, during, and after experimental stimulations and lesions were made at specific sites within the centers and pathways of pupillary control.

Pharmacodynamic experiments were done on patients with the syndrome under consideration, as well as on normal human subjects and on animals in connection with experimental lesions or stimulations.*

* From the Department of Ophthalmology (Laboratory of Pupillography), Columbia University College of Physicians and Surgeons, and the Institute of Ophthalmology, Presbyterian Hospital. This work was supported by the Harriman Fund and by a grant from the U. S. Public Health Service (B-253 C3, Sens. Dis. 5).

* A double-lens, 35-mm. camera has been used in this laboratory for the past[4, 4a, 4b, 4c] 11 years, in order to cut out the interocular space and to obtain large pictures (one-half natural size) of maximal possible clarity. An electronic measuring apparatus employing a rotating mirror, photocell, and oscilloscope has been used for measurements.

The clinical conditions associated with the 130 cases of fully developed Argyll Robertson syndrome considered in this paper included tabes dorsalis, cerebral syphilis, dementia paralytica (together 51.6 percent), multiple sclerosis (10.3 percent), virus infections (9.5 percent), heredodegenerative conditions (7.9 percent), arteriosclerosis cerebri (10.3 percent), post-concussion syndromes (3.2 percent), and other conditions (7.2 percent). The additional group of 100 clinical cases of initial Argyll Robertson syndrome were mainly, but not exclusively, associated with the clinical pictures of various kinds of syphilis of the central nervous system, multiple sclerosis, and virus infections.

II. Miosis

The mechanisms of miosis were recently analyzed;[3] I shall review them briefly.

The definition of miosis varies greatly from author to author; it was therefore necessary to define the term. Since the size of the normal pupil varies with the intensity of illumination, the degree of near vision, the emotional status of the subject, the age of the subject, and other factors, any statement involving pupillary size can be meaningful only when the conditions under which the measurements were taken are specified.

It was found that after at least six minutes of adaptation to darkness the variability according to age is much less than generally assumed as long as near vision and disturbing sensory stimuli or emotional upsets are excluded. Children and adolescents up to 20 years showed values between 7.0 and 9.0 mm.; adults from 40 to 60 years between 6.8 and 8.0 mm. The values varied from 5.8 to 7.5 mm. between 60 and 70 years and from 5.5 to 7.5 mm. between 70 and 80 years. We therefore arbitrarily termed "miotic" such pupils which independent of age show a diameter of less than 5.0 mm. after at least six minutes of adaptation to darkness. Our values are from 1.0 to 3.0 mm. larger than the ones which had been determined clinically in the same patient.

The mechanisms by which various types and degrees of miosis are produced are best explained by demonstrating the effects of lesions at different sites of pupillary centers and pathways. This is done in Figure 1 which shows the size of pupils and the shapes of the pupillary reflex to light under the various conditions of innervation which cause miosis. The pupils were adapted to darkness which was interrupted by light stimulations (one second) at F, G, H, and I (fig. 1).

A. Figure 1-F shows for comparison the shape of the normal reflex to light. The pupil contracts after a latency period of about 0.2 sec.; the speed of contraction increases and reaches its peak at about midway through the contraction and diminishes slowly. This is shown in the C-wave of the differential curve, which is the curve of variations of speed at which contraction and redilatation take place (lower section of fig. 1). The redilatation goes on in two waves (D- and E-waves) of increasing and decreasing speed.

The variations of speed as shown in the differential curve give the reflex shape most of its characteristic features.

B. When the sympathetic chain is interrupted by a clinical lesion in man or experimentally in animals, the pupil becomes slightly smaller than normal. In the case of Figure 1-G, the diameter of the sympathectomized pupil measured about 5.8 mm. (instead of a normal diameter of 7.0 to 8.0 mm. before sympathectomy). The sympathectomized pupil contracts to light at an even higher speed than normal (C-wave of differential curve), while the second phase of

With this instrument 7,200 pupils can be measured per hour with less than two-percent error. During the examination the patient sits in a comfortable reclining chair in a dark room with his head supported by a headrest and a chinrest. To prevent convergence, he looks at a small dark-red point of light at apparent infinity. His eyes are illuminated by dark-red infrared lights (tungsten, 6 V, 2.5 A, through Wratten 88A filters).

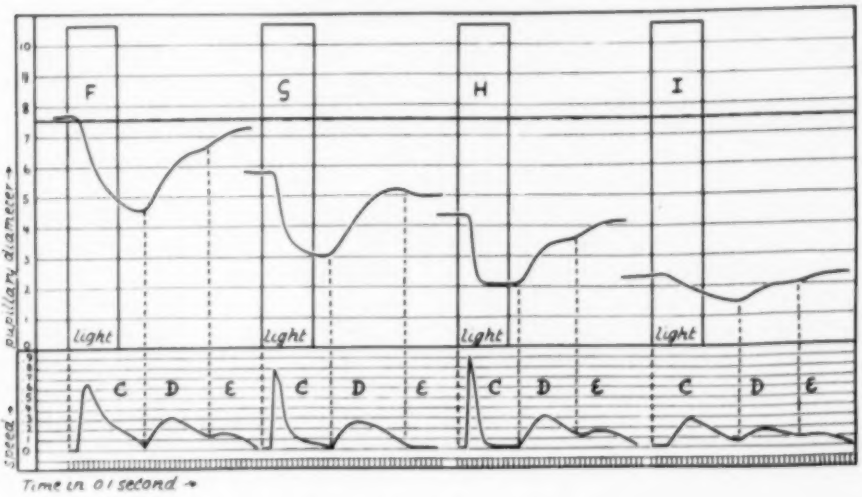

Fig. 1 (Lowenstein). *Miosis and pupillary reflex to light.*

The solid horizontal line shows the average width of the normal pupil (between seven and eight mm.) after adaptation to darkness in persons up to 60 years of age.

The upper section shows the original pupillograms F, G, H, and I, in which the pupillary diameter (in mm.) is plotted against time in 0.1 second. All pupils were under the condition of darkness; the light stimulations at F, G, H, and I lasted 1.0 second each.

The lower section shows the differential curves of the contractions and dilatations of the light reflex in F, G, H, and I. The differential curves show the speed (in mm. per second) at which pupillary contraction and dilatation occur. The differential curve of the normal contraction to light and its subsequent redilatation consists of three waves referred to as C-, D-, and E-waves.

F—Reaction of the normal pupil to light with its differential curve beneath. In this case the pupil had a diameter of 7.6 mm. in darkness and contracted to about 4.5 mm. under the influence of the light stimulus F.

G—When the sympathetic cervical chain was interrupted the pupil became slightly miotic (diameter 5.8 mm.), reaction to light showed a higher speed (as seen in the higher C-wave of the differential curve), and reached the peak of speed earlier than the normal reaction F. The E-wave of the differential curve is absent.

H—A unilateral lesion anterior to the third nerve nucleus which interrupts supranuclear fibers from cortex and diencephalon caused in this case a diameter of the pupil of only 4.4 mm. Contraction to light shows characteristic tonohaptic (square) shape; the speed of contraction was even higher and peak speed reached earlier than in reaction G. D- and E-waves of differential curves are normal.

I—A spastic pupil with strong miosis obtained by stimulation of the third nerve nucleus or instillation of pilocarpine or physostigmine into the conjunctival sac. The pupil became extremely miotic (2.3 mm.); the reaction to light became sluggish (the C-wave of the differential curve was low) and inextensive. D- and E-waves are present but low.

redilatation (E-wave of differential curve) is absent. The sympathectomized pupil dilates when homatropine is instilled into the conjunctival sac, fails to dilate on instillation of cocaine hydrochloride (two percent), but does dilate when adrenalin is instilled in addition to cocaine. Dilatation to psychosensory stimuli is sluggish and reduced.

C. Figure 1-H shows the effect of a unilateral lesion in the midbrain anterior to the third nerve nucleus. The Westphal-Edinger nucleus is thereby deprived of its supranuclear inhibitory impulses from diencephalon and cortex, and the pupillary reactions show characteristic release phenomena: the pupils become bilaterally miotic; their diameters range from about 3.0 to 5.5 mm. In the case of Figure 1-H the diameter measured 4.3 mm. The reactions to light adopt a characteristic "square" shape, which I

called "tonohaptic"; the contraction speed is much greater and the peak of speed reached earlier than normal, while the redilatation remains fairly normal. The pupils dilate normally to psychosensory stimuli, to cocaine and homatropine, and there is no "paradoxical" dilatation to adrenalin.

D. If the third nerve nucleus is stimulated the pupil may become very small. Its diameter measured 2.3 mm. in the case of Figure 1-I. This strong miosis is due to spastic contraction of the sphincter muscle. Light stimuli cause the miotic pupil to contract sluggishly and inextensively. Even these sluggish and inextensive contractions show variations of speed, as illustrated by the presence of the three waves in the differential curve (fig. 1-I, C-, D-, and E-waves). Owing to the spastic sphincter contraction the miotic pupils usually show somewhat reduced dilatation to psychosensory stimuli. They dilate up to normal values with cocaine hydrochloride and they may become as large as the normal pupil when atropine or homatropine is instilled. This extensive atropine dilatation shows the cholinergic nature of this type of miosis. This type of miosis is characteristic of that seen in the Argyll Robertson pupil.[3]

III. Pupillary dilatation and mydriasis

In order to make the mechanism of the Argyll Robertson syndrome more understandable it is necessary to briefly discuss pupillary dilatation. The pupil dilates, of course, and mydriasis develops when the third nerve is damaged or cut. In addition, at least three mechanisms are known by which the pupil may dilate under normal and pathologic condition. These are dilatation due to (1) sympathetic stimulation, (2) parasympathetic inhibition, and (3) neurohumoral mechanisms due to hypersensitivity of denervated structures. They are illustrated in Figures 2-A and B.

Figure 2-A (broken line) shows normal

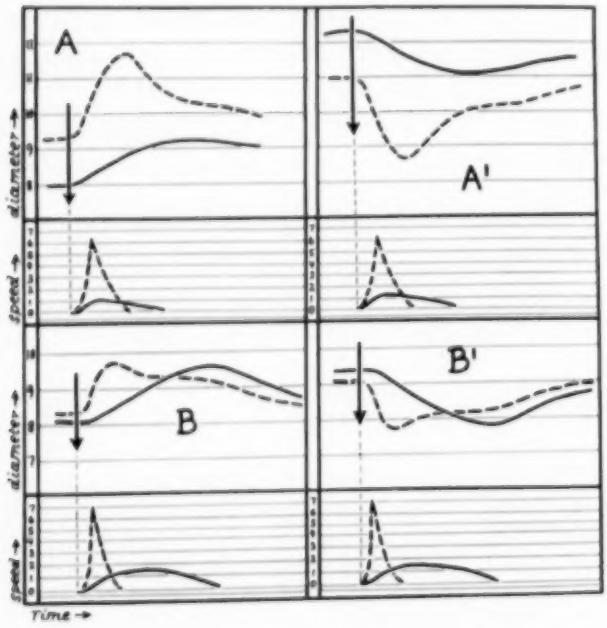

Fig. 2 (Lowenstein). *Mechanisms of pupillary dilatation.*

A—Broken line: Normal pupillary dilatation to a short psychosensory stimulus. Differential curve (only the dilatation part is shown) resembles the differential curve of pupillary contraction to light.

A—Solid line: Dilatation to psychosensory stimulation immediately after cervical sympathectomy. Slow dilatation movement with a type of differential curve which is different from the differential curve of its normal fellow pupil, slowly ascending curve with a flat roof.

B—Broken line: Effect of psychosensory stimulus of somewhat longer duration in a normal pupil. Differential curve is similar to the one of normal contraction to light.

B—Solid line: Dilatation of sympathectomized pupil which has become hypersensitive to adrenalin. The hypersensitive pupil reaches its peak of dilatation very slowly and at even speed at a time when the normal pupil has long started to recontract, so-called "paradoxical dilatation." Differential curve shows slow ascending branch and a long, flat roof.

A' and B' show the mirror images of Figures A and B. These mirror images resemble certain types of normal and clinically significant contractions to light or near vision.

pupillary dilatation to a short psychosensory stimulus (mechanism 1). Its differential curve shows the same type of increasing and decreasing speed as the normal contraction to light. Figure 2-A (solid line) shows the pupillary dilatation response to a short psychosensory stimulus on a sympathectomized pupil in the same case. This reaction is due to inhibitory effects running over the third nerve (mechanism 2). The movement shows a different type of differential curve; a slow increase of speed is followed by a slow decrease.

Figure 2-B (solid line) shows the effect of a psychosensory stimulus after the pupil had become hypersensitive to adrenalin some time after sympathetic denervation (mechanism 3). The pupil dilates slowly, at a constant speed. The peak of dilatation is reached when the non-sympathectomized pupil is already in the process of recontracting (so-called "paradoxic reaction"). The differential curve shows slow increase of speed, a long period of constant speed, and slow decrease. This neurohumoral type of dilatation is of long duration.

As early as 1911, M. Lewandowsky[5] (page 419), referring to extensive experiments particularly by Anderson,[6] stated:

"The activity of the oculomotor nerve shows in all its features the mirror image of sympathetic activity. As there is paradoxical dilatation in the sympathetic system, there is paradoxical constriction in the oculomotor system."

This statement is illustrated by Figure 2-A′, which is drawn as mirror image of Figure 2-A, and by Figure 2-B′, which is drawn as mirror image of Figure 2-B. The broken line in Figure 2-A′ represents a type of contraction which occurs frequently and under various normal conditions; the solid line in Figure 2-A′, which is the mirror image of a pathologic dilatation type, namely the one occurring after sympathectomy, resembles pupillary contraction to light in cases of partial lesion of the third nerve (fig. 10) if the pupil is mydriatic (diameter of 7.5 mm. or more); it resembles contractions to light of spastic pupils in cases of miosis (fig. 3). This resemblance, however, is only superficial because the dynamic structure as shown by the differential curve is neither the one of partial lesion of the third nerve (fig. 10) nor the one of spastic miosis (fig. 3-B).

The same is true of the reaction represented by the solid line of Figure 2-B′ which is the mirror image of a hypersensitive reaction after sympathectomy. It resembles superficially the reactions to light of mydriatic pupils with third nerve lesions or of miotic spastic pupils. The dynamic structure of those movements again is different.

A more complete analogy than that suggested by Lewandowsky between sympathetic and parasympathetic pupillary pathologic process becomes evident when comparison is made not only of the sequelae of complete denervations (full sympathectomy and full section of third nerve) but of partial damages to either nerve as well.

The differential curves of these movements illustrate an important fact; namely, that normal pupillary reflex dilatation shows the same dynamic structure, that is, the same type of increase and decrease of speed, found in the normal contraction movement of the reflex to light. These movements however, which appear after sympathectomy and which are due to parasympathetic inhibition or to neurohumoral mechanisms, show characteristically altered differential curves.

IV. Pupillographic picture of the Argyll Robertson syndrome*

Cases with the Argyll Robertson syndrome, either bilateral or unilateral (figs. 3A and 3B), show all the characteristics of parasympathetic spasm without damage to sympathetic centers or pathways. The psychosensory dilatation is present (fig. 6) but possibly diminished according to the degree of parasympathetic spasm. The miosis seen

* I refer expressly to my paper "Miosis in Argyll Robertson, etc."[8]

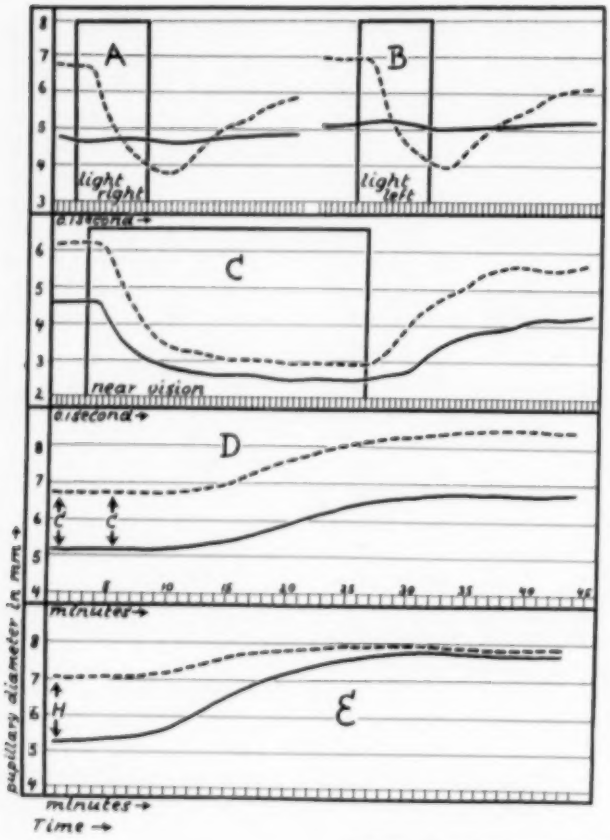

Fig. 3A (Lowenstein). *Case of unilateral Argyll Robertson syndrome.*

A—Reaction to light when the pupil (broken line) was stimulated.

B—Reaction to light when the left, miotic, pupil (solid line) was stimulated.

C—Reaction to near vision. Both pupils contract at about the same rate. The miotic pupil redilates at a slower rate. Anisocoria persists at the peak of contraction.

D—Reaction to cocaine hydrochloride, two drops of two-percent solution into the conjunctival sac, at arrows. The left, miotic, pupil dilates at the same rate as the normal right pupil, showing that the sympathetic supply is unimpaired.

E—Reaction to homatropine hydrochloride (two percent), one drop, at arrow, into the conjunctival sac. The miotic pupil dilates to about 7.8 mm. It becomes equal to its fellow pupil; anisocoria disappears at the peak of dilatation, indicating that the miosis is cholinergic in nature.

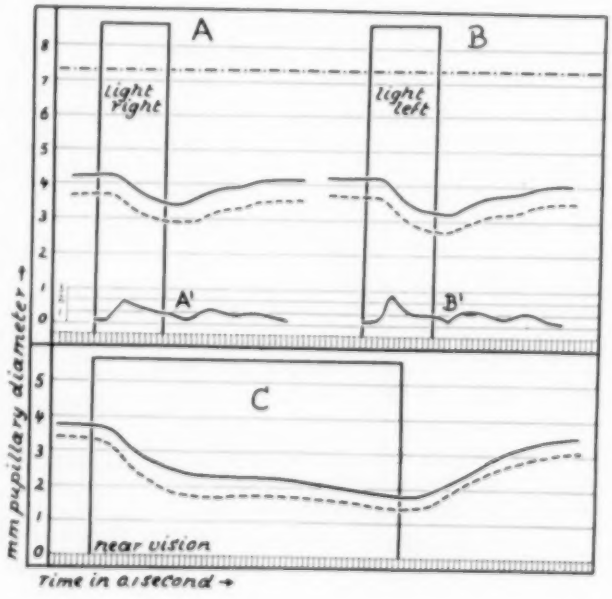

Fig. 3B (Lowenstein). *Incomplete bilateral Argyll Robertson syndrome.*

Both pupils are miotic; diameter of right pupil (solid line) measures 4.1 mm., diameter of left pupil (broken line) 3.7 mm., after adaptation to darkness.

The contraction to light is less than one mm. when the right eye is stimulated and about one mm. when the left eye is stimulated; the reaction to near vision is about two mm.

Differential curves (A', B') show that the reactions are neural, not neurohumoral, in nature.

in these cases is cholinergic in nature since it disappears after instillation of parasympatheticolytic drugs such as atropine or homatropine (fig. 3-A). The features of the miosis found in Argyll Robertson pupils differ in no way from miosis artificially produced in normal pupils, either peripherally, by instillation of parasympatheticomimetic drugs such as physostigmine and pilocarpine (fig. 4, also[3] page 7), or centrally, for example by deep barbiturate anesthesia (fig. 5—first reaction to light).

The miosis seen in barbiturate anesthesia can be overcome. Stimulation (fig. 5—between the arrows) of the inhibition pathways to the third nerve nucleus dilates the pupil. At the end of the stimulation the light reflexes no longer show the sluggishness present during the spastic condition (fig. 5—reactions I, IV, V, and VI).

Miosis in Argyll Robertson pupils, then, is due to parasympathetic spasm with the final motor path to sphincter and dilator pupillae unimpaired.

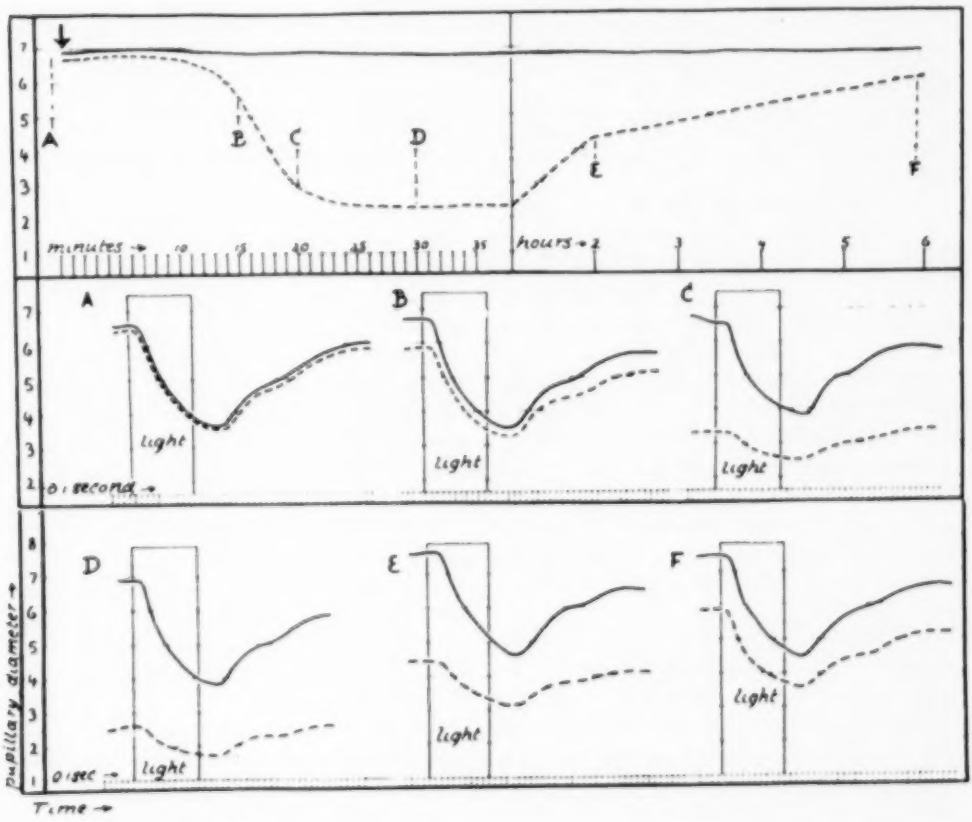

Fig. 4 (Lowenstein). *Pilocarpine miosis.*

First line: Contraction of pupil after instillation of one drop of a one-percent solution of pilocarpine hydrochloride (arrow) into one conjunctival sac. A, B, C, D, E, and F indicate the moments when light reflexes were elicited during constriction and redilatation.

Second and third lines: With increasing miosis the pupillary reaction becomes more sluggish and less extensive but the reactions to light maintain the characteristics of neural nature.

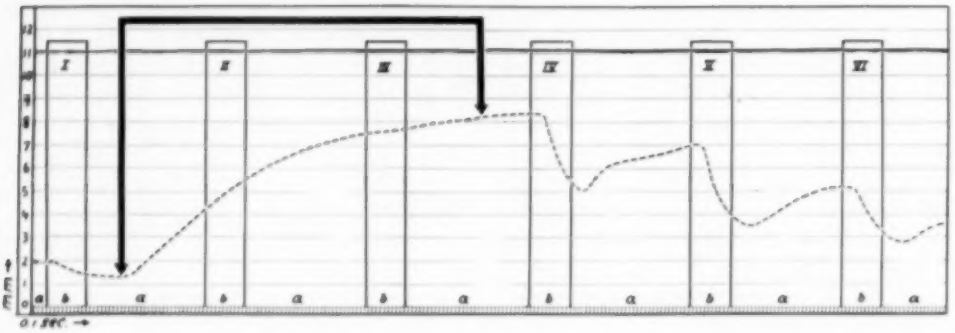

Fig. 5 (Lowenstein). *Miotic pupil (of two mm.) due to deep barbituate anesthesia (cat).*

1. Reaction to light is sluggish and inextensive.

2. Between the two arrows: Electrical stimulation in the dorsal part of the interbrain (area "B"). The inhibition pathways to the third nerve nucleus are stimulated and the pupil dilates slowly, but not completely (to about eight mm.). Light stimuli (II, III) during the process of dilatation do not cause the pupil to contract.

3. After the end of electrical stimulation, pupil recontracts slowly. Light stimuli during the process of recontraction (IV, V) result in almost normal reactions to light.

4. Reactions to light (VI) become increasingly spastic as the pupil becomes more miotic.

V. Dissociation between near vision and light pupillary reflexes

The next feature of the Argyll Robertson syndrome to be explained is the dissociation, that is, the absence of the reflex to light in the presence of near vision contraction. It might be mentioned parenthetically that pupillographic records of clinical Argyll Robertson syndromes show the pupillary reactions to near vision sometimes active, but never hyperactive, and frequently hypoactive. The sometimes reported "hyperactivity" is only a relative one, namely, in comparison with the extent and rate of the reflex to light.

1. The dissociation observed clinically is in itself evidence of the fact that, at least at one point within the reflex arc, either the centers, the pathways, or the effector organ for the two reactions must be separate. The usual dissociation consists of the absence of light reflexes in the presence of near vision reactions. But the reverse is also seen; the light reflexes may be present and near vision reactions absent. Such findings are not at all as rare as is generally assumed in the literature. However, there are diagnostic difficulties inasmuch as one is never sure to what degree a convergence effort has really been made by the patient. This difficulty is eliminated in cases of unilateral absence of pupillary contraction to near vision.

Figure 6 shows the pupillogram of a patient who had suffered from a virus infection two years prior to examination. The diameter of his right pupil (solid line) measured 5.3 mm. after adaptation to darkness, his left pupil 3.1 mm. The right pupil showed prompt and extensive direct and indirect reactions to light; the left pupil (broken line) reacted neither directly nor indirectly to light. Psychosensory dilatation was present on both sides; the dilatation amounted to about 2.0 mm. on the right side and 1.0 mm. on the miotic side.

The right pupil, which reacted well to light, did not react at all to near vision. The left pupil, which was immobile to light, contracted about 1.5 mm. to near vision.

The patient showed a typical Argyll Robertson pupil on the right side and an "inverse Argyll Robertson syndrome" on the left side.

2. It is frequently assumed that the con-

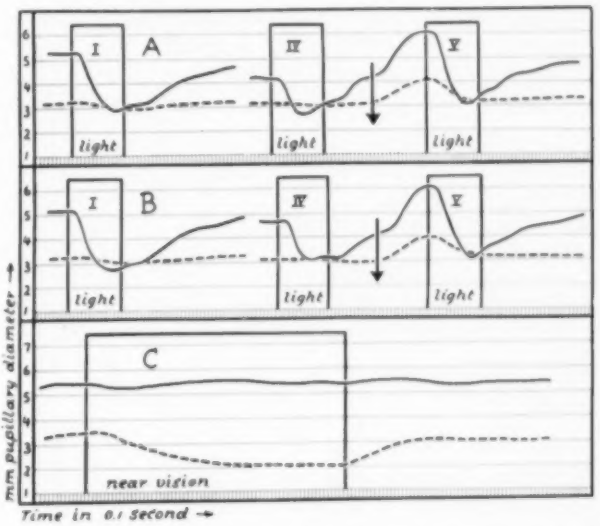

Fig. 6 (Lowenstein). *Dissociation between contraction to light and contraction to near vision.*

Case of a 42-year-old man with a virus infection two years prior to examination. Typical Argyll Robertson of the left pupil; inverse Argyll Robertson of the right pupil.

A—The right pupil (solid line) reacts well to light, both directly (A I, IV, and V) and indirectly (B I, IV, and V), and to psychosensory stimuli (arrow); it shows good psychosensory restitution (reaction V is much more extensive and of higher speed than reaction IV), but the reaction to near vision (C) of the right pupil is absent *(inverse Argyll Robertson syndrome)*.

B—Stimulation of the miotic left pupil (broken line) by light shows almost no pupillary reaction (0.2 mm.) on the ipsilateral, but normal reaction (about 2.3 mm) on the contralateral side. Psychosensory dilatation and restitution (arrow) of the left pupil are present. Reaction to near vision also is present in the left pupil *(typical Argyll Robertson syndrome)*.

traction impulses to the iris upon near vision are stronger than those due to light stimulation. This assumption is not correct. It is true that a pupil, contracted by light, contracts further when near vision impulses are added —as long as the individual is not blinded by the light stimulus and therefore unable to fix a near point. But it is equally true that a pupil contracted by near vision contracts further when a light stimulus is added. In Figure 7, a 22-year-old subject adapted to the illumination of a dimly lit room showed pupillary contraction to near vision (a). Light stimuli (fig. 7 b^1, b^2) to the eyes during convergence elicited further contractions and redilatations with normal light reflex shapes. It is there-

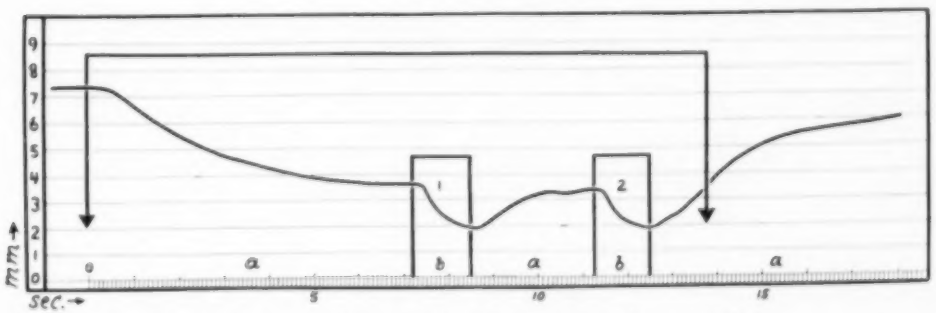

Fig. 7 (Lowenstein). *Pupillary reaction to light in addition to near vision contraction.*

A young woman, aged 22 years, without pupillary pathology and in good general health.

Contraction to near vision (between the two arrows) in a dimly lighted room. Pupils contracted to about 3.5 mm.

Additional stimulation by light (b^1 and b^2) of the eye in convergence results in additional contraction movements of the miotic pupils.

In a, a, a—both eyes are in half darkness (dimly lighted room); in b, b—stimulation of the eye by light.

fore inadmissible to explain the dissociation between these two reactions by supposing damage just strong enough to eliminate the light reflex, but too weak to suppress the supposedly stronger contraction to near vision. Both are of about equal strength, in younger individuals; in older persons the light reflex is usually more powerful.

3. The three components of near vision, namely, accommodation, convergence, and pupillary contraction, have separate centers within the third nerve nucleus and separate efferent paths.

In recent experiments we stimulated the third nerve of monkeys (Macaca mulatta) intracranially with the aid of a stereotaxic instrument. A bipolar electrode was placed on the medial side of the third nerve at a level slightly posterior to the optic chiasm. The following sequence of events could be observed:

a. On low intensity stimulation (1.0 to 2.5 volts) the lens was pushed forward and caused the ipsilateral iris to bulge forward. In doing so, the pupil was dilated about 1.0 to 2.0 mm. This appeared possible because the non-contracted iris did not offer the resistance necessary to prevent the bulging.

b. When the voltage was slightly increased (to about three to five volts), the ipsilateral internal rectus contracted rhythmically with the stimulus. No active pupillary movement was visible. The bulging of the lens was still present, but the pupil was no longer dilated by its pushing.

c. When the voltage was further increased (to about five to eight volts), a very active contraction of the pupillary sphincter occurred, despite vigorous pushing by the lens. At this voltage the strong pupillary contraction on the ipsilateral side was accompanied by slight contraction of the contralateral pupil but not of the contralateral ciliary body and internal rectus. This participation of the contralateral pupil could easily be explained by spreading of the current into the neighboring optic chiasm and the afferent pathways of the reflex to light.

d. When the electrode was lowered 1.0 mm., active pupillary constriction occurred upon stimulation by a lower (three to five) voltage; bulging of the lens no longer produced pupillary dilatation.

Comparable results were observed as far back as 1878 by Hensen and Völckers[7] upon stimulation of the ciliary ganglion and in the posterior part of the wall of the third ventricle, and more recently by Bender and Weinstein[8] who stimulated the third nerve nucleus about 1.0 mm. below the focus for pupillary constriction and 1 mm. above the focus for downward and inward movements of the globe.

4. When the ciliary ganglion of our monkeys was nicotinized, contraction of the internal rectus muscle was the only remaining effect of intracranial stimulation of the third nerve. The absence of both pupillary constriction and accommodation proves that all cholinergic fibers from the third nerve to ciliary body and iris undergo synapses in the ciliary ganglion or possibly existing accessory ciliary ganglia.

These experiments by no means prove the existence, in the third nerve, of separate fibers for pupillary contraction to light on one hand and for pupillary constriction to near vision on the other hand. But they lead necessarily to the conclusion that the nervous impulses which cause accommodation, convergence, and pupillary constriction must arise from independent cell groups in the third nerve nucleus. Accommodation, convergence and pupillary contriction are associated, synchronized, and controlled by supranuclear connections, but they are not mechanically caused in the periphery by one another.

The site of synapsis for pupillary constriction to light within the third nerve nuclear complex is according to present knowledge the Westphal-Edinger nucleus; according to Warwick[9] the site of synapsis for near vision reactions is not, as hitherto assumed, the nucleus of Perlia, but some other (unknown) group of cells close to the Westphal-Edinger

nucleus. The efferent fibers from both these cell stations synapse in the ciliary ganglion. It is unknown whether or not the postganglionic fibers leave this ganglion as discrete and functionally independent fibers.

The dissociation between light reflex and contraction to near vision in Argyll Robertson pupils must be explained on the basis of separate centers or pathways for the two functions.

What are we, then, able to say about the site of the lesion which results in the Argyll Robertson syndrome?

1. The iris itself cannot be the site of the causative lesion. If atrophy of the iris had progressed far enough to make the contraction to light impossible, it would make all other pupillary movements equally impossible. But contraction to near vision, dilatation to psychosensory stimulation, to cocaine, and to atropine are present.

2. The supposition of atrophy of only one of the two iris muscles cannot solve our problem. The dilator muscle is unimpaired, since dilatation to cocaine and to psychosensory stimuli is preserved; moreover, even complete sympathectomy would cause a dark-adapted pupil to contract only from about 8.0 mm. to about 6.0 mm., that is, it would cause only a relative and not an absolute miosis.

3. Atrophy of the sphincter muscle alone would not explain the phenomenon. The pupil does contract to near vision. Moreover, atrophy of the sphincter would produce a mydriatic and not a miotic pupil.

4. Neither can the nerve nets nor the endings within the iris be the site of the responsible lesion. These nets are so interwoven that complete destruction of one part must necessarily involve the other. Again both near vision contraction and light reflex would be lost, and a mydriatic, not a miotic, pupil would result.

5. The ciliary ganglion and its efferent fibers were frequently considered to be the site of the lesion (as by Marina[10] in 1901). This theory is without basis, since destruction of the ciliary ganglion leads to mydriasis and not to miosis. Anatomic research on cases of tabes dorsalis with Argyll Robertson pupils (Thomas,[11] 1912, and by Rizzo,[12] 1920, and others) showed the ciliary ganglion, the third and the fifth nerves, and the short and long ciliary nerves unimpaired.

Extirpation of the ciliary ganglion or interruption of cholinergic fibers (Anderson 1905, Shen and Cannon, 1936, Lissák and Martin 1940, and many others) increase the sensitivity of the denervated iris sphincter to parasympatheticomimetic substances; simultaneously it decreases the concentration of the destructive cholinesterase at the nerve terminals.

It should therefore be expected that destruction of the ciliary ganglion leads to mydriatic pupils and hypersensitivity of the sphincter to cholinergic discharges. In this connection I refer to an interesting pupillographic picture (fig. 8).

Both pupils had almost equal size after adaptation to darkness, the right pupil (solid line) being only slightly larger than the left one (broken line). The right pupil reacted to light very sluggishly, inextensively, and only after a long latency period, both on direct stimulation (fig. 8-A) and on indirect stimulation (fig. 8-B). The reaction to near vision of the left pupil was not extensive but normal in shape. The near vision reaction of the right pupil was slightly more extensive; it showed all the characteristics of a "hypersensitive" reaction: a long latency period and long-lasting slow contraction which goes on at almost constant speed throughout the movement. This contraction is the exact mirror image of the pupillary dilatation of the sympathetically denervated iris which has become hypersensitive to adrenergic substances, as seen by the superimposition in Figure 8-E of the mirror image of the hypersensitive reaction of Figure 8-D upon the contraction curve of Figure 8-C.

The contraction to near vision shown here is found in clinical cases of Adie's syndrome and the curve comes from a 42-year-old

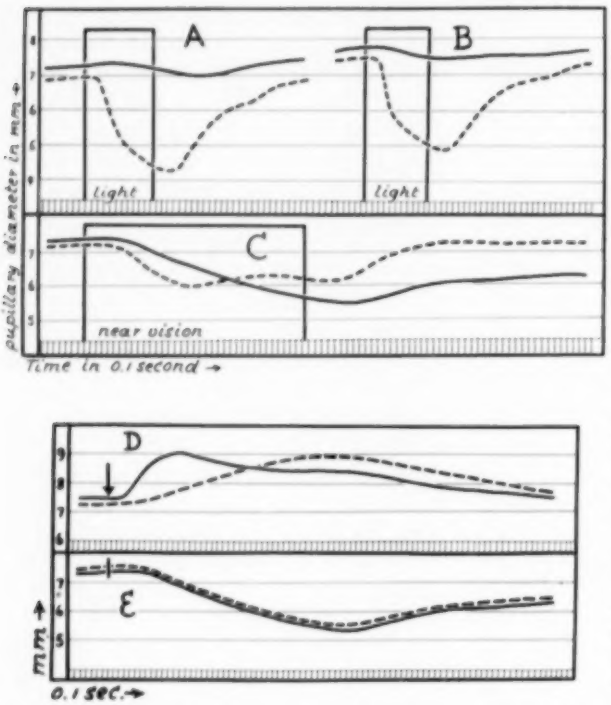

Fig. 8 (Lowenstein). *Unilateral Adie syndrome in a 42-year-old woman.*

A—The right pupil (solid line) shows only traces of reactions to light, both directly (A) and indirectly (B). The remnant reaction is very sluggish, shows a long latency period of more than 0.3 second, and lasts longer than the light stimulus.

B—The left pupil (broken line) reacts almost normally to light, both directly and consensually.

C—Reactions to near vision:

Reaction of the left pupil to near vision (broken line) is normal in shape but it is not extensive.

Reaction of the right pupil to near vision (solid line) shows a long latency period, a sluggish long-lasting contraction of constant speed, and a slow redilatation.

D—Dilatation to psychosensory stimulation in a unilateral sympathectomized cat in which hypersensitivity to adrenalin existed (broken line) (refer to fig. 2B). The solid line represents the normal dilatation of the nonsympathetomized, normal pupil of the same animal.

E—Mirror image of the broken line of D and beneath, reduced to equal dimensions, the contraction to near vision of the Adie pupil (solid line). It is evident that both movements are completely alike.

woman suffering from it. The reaction shape is typically neurohumoral; it suggests that the site of the lesion in Adie's syndrome may be within the neuron starting in the ciliary ganglion. This conclusion is in agreement with recent anatomic work done by Friedrich Ruttner[12] who found the ciliary ganglion destroyed in a case of Adie's syndrome. However, here I am not dealing with Adie's syndrome, but with the Argyll Robertson pupil. The contraction to near vision in Argyll Robertson pupils shows none of the characteristics of hypersensitivity. Both contraction and redilatation to near vision show the characteristics of neural, not neurohumoral reactions (fig. 9). The ciliary ganglion

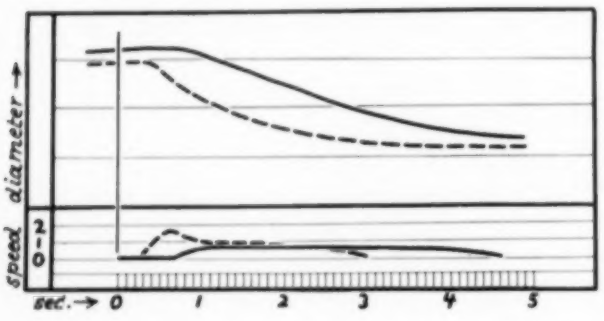

Fig. 9 (Lowenstein). *Typical contractions to near vision in a case of Argyll Robertson syndrome (broken line) and Adie syndrome (solid line).*

At first glance both movements do not appear to be very different. But the differential curve shows their dynamic structures to be typically different, the Adie contraction showing all the signs of a sluggish, long-lasting neurohumoral reaction, the Argyll Robertson pupil all signs of a sluggish, but neural, reaction (differential curves in lower section).

therefore cannot be the site of the damage.

6. Destruction or damage in the third nerve nucleus or the efferent third nerve between its nucleus and the ciliary ganglion results in mydriatic pupils (fig. 10). In case of complete destruction no reaction to light can be elicited. In case of partial damage the mydriatic pupil shows sluggish and inextensive reactions to light and near vision. No signs of cholinergic iris spasms are present. The reactions to psychosensory stimuli are preserved and the remnant reactions to light show in their dynamic structure all signs of neural, not neurohumoral or inhibitory mechanisms.

7. Incomplete destruction of the optic nerve results in quantitative reduction of the pupillary reflexes to light and to darkness and the appearance of low intensity reactions upon stimulation by light of "standard" (15 f.c.) intensity. Since the reflex to near vision is unimpaired in these lesions, a pseudo-Argyll Robertson syndrome[14] may develop.

This is shown in Figure 11 in which the right optic nerve was damaged by previous retrobulbar neuritis in a case of multiple sclerosis. The light reactions elicited by stimulation of the right eye show responses of normal shape. The contraction of the directly reacting right pupil and the indirectly reacting left pupil is about half as extensive as the reactions of both pupils to near vision and of both pupils to light when the left eye is stimulated.[14]

Such a syndrome, particularly when it is bilateral, may clinically look like an incomplete Argyll Robertson. It can be easily distinguished pupillographically—not only because these pupils are mydriatic in darkness and not miotic, but also because of their normal, although low intensity, reaction shape, the absence of deformations of the pupil, and the absence of anisocoria under all conditions of light and darkness. In these cases anisocoria is present only if there is additional involvement of other neurons of pupillary control.

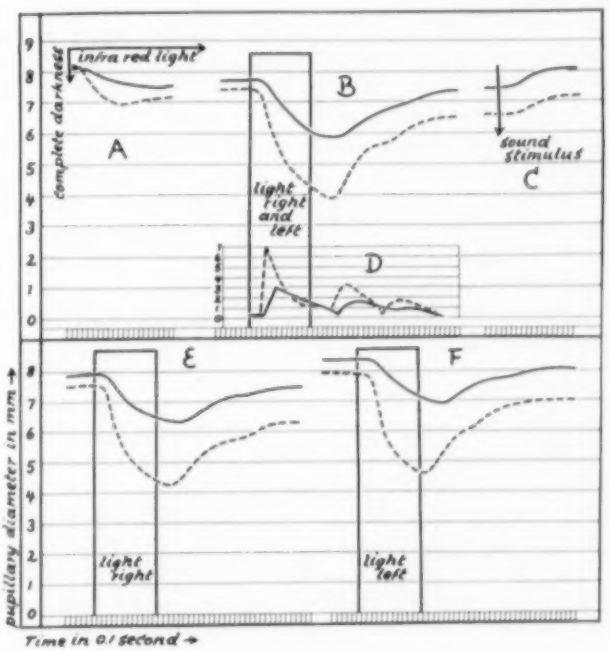

Fig. 10 (Lowenstein). *Unilateral third-nerve condition in a case of multiple sclerosis.*

After adaptation to darkness and infrared light the right (solid line) and left (broken line) pupils show almost the same size.

A—The right pupil (solid line) is not only less responsive to white light but also to dark red infrared light.

B—Reaction to light on simultaneous stimulation of both eyes. The right pupil reacts with sluggish and inextensive movements; reaction of the left pupil is normal.

C—Reactions of both pupils to a sound stimulus. Reactions of both pupils are about equal.

D—Differential curves of the light reactions shown in B. The movements show quantitative differences but equal dynamic structure. They are neural, not neurohumoral, in nature.

E—Reactions of both pupils to light on stimulation of the right eye.

F—Reactions of both pupils to light on stimulation of the left eye.

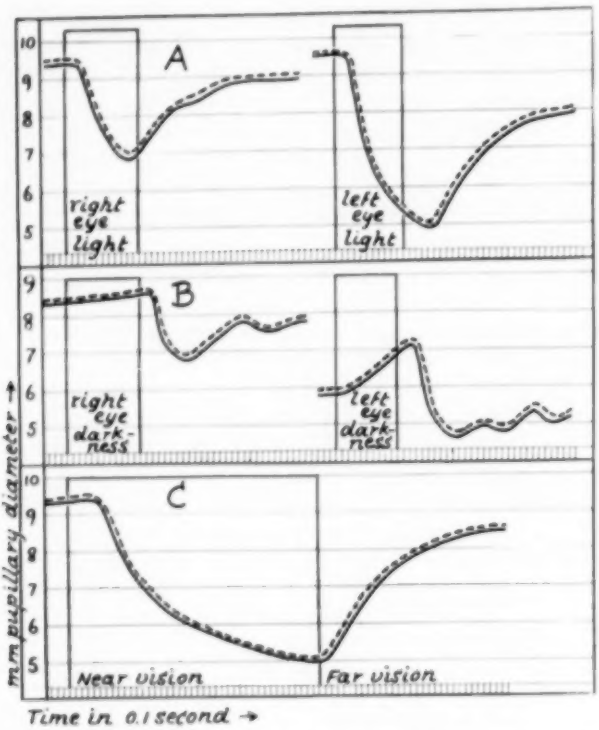

Fig. 11 (Lowenstein). *Phenomenon due to unilateral lesion of one (right) optic nerve.*

A—Stimulation of the right pupil (solid line) by light of standard intensity results in low-intensity but otherwise normal reaction shapes on both sides.

Stimulation of the left eye with light of the same intensity results in bilaterally normal reactions; its extent is almost double that of the other side.

B—Reaction to darkness shows the first dilatation phase diminished on the right side, but normal on the left side.

C—Reaction to near vision shows about the extent of the reactions to light on the left side. This is not an incomplete Argyll Robertson of the right side in spite of the dissociation between the reaction to light on the right side—which has a low-intensity reaction—and the reaction to near vision on both sides which have normal extent.

If this lesion includes both optic nerves, the resulting phenomenon may be called a pseudo-Argyll Robertson syndrome.

8. As shown before, stimulation of the Westphal-Edinger nucleus may produce a miotic pupil which reacts to light only sluggishly and inextensively, if at all. I mentioned before that a similar miosis easily can be produced for any desired length of time by local application of parasympathetic-conimetics (fig. 4) or by deep barbiturate anesthesia (fig. 5). The reactions to light of the resulting miotic pupils show reflex shapes similar to the remnant reflexes in Argyll Robertson pupils. The dynamic structures of the movements in all these miotic pupils are those of neural, not of inhibitory or neurohumoral mechanisms.

The analysis of the shape of pupillary contraction to near vision in cases of Argyll Robertson pupils, unlike the near vision reactions in Adie's syndrome, shows *neural* and not *neurohumoral* features.

We see, then, that miosis in Argyll Robertson pupils is due to cholinergic sphincter spasm and that the dissociation between light and near vision reactions has to be explained on the basis of separate pathways or centers somewhere in the reflex arcs. Where could a lesion causing such a picture be situated?

The afferent pathways of the light reflex meet at the Westphal-Edinger nucleus with a highly sensitive pupillomotor effector system. Light of very low intensity (or in experimental animals currents of very low voltage) can set this system into motion. A lesion which would deafferentiate the Westphal-Edinger nucleus would further increase the sensitivity of this central synapse toward its transmitter substance which is most likely to be acetylcholine.[15]

This assumption is in agreement with what Cannon called a "law of denervation."[16] He formulated it (1939) in the following way:

"When in a series of efferent neurons a unit is destroyed, an increased irritability to

chemical agents develops in the isolated structure or structures, the effects being maximal in the part directly denervated."

In 1948, Drake and Stavraky[17] showed that the principle of sensitization by denervation holds true not only in regard to the influence of higher over lower levels in the central nervous system, but is applicable also to one and the same neural level and that sensitization occurs by denervation within a reflex arc. As a matter of fact, it embraces all the synaptic terminations within the central nervous system, regardless of their efferent or afferent nature.

In 1953 Teasdall and Stavraky[18] reported that section of the posterior nerve roots increases the excitability of the deafferentiated spinal neurons to nerve impulses reaching them via the corticospinal tract, this taking place after an initial depression of spinal activity.

Acetylcholine is released on preganglionic nerve fibers and stored at their endings; degeneration of preganglionic fibers is followed by disappearance of acetylcholine, of cholineacetylase, and true cholinesterase (Feldberg). But the ganglion cells which are the effector structures on which the released acetylcholine acts become hypersensitive.

This would be the end of the story if we were dealing with some peripheral ganglion, as in the case of the ciliary ganglion in the Adie's pupil. The denervated pupil would be large and contract only when, in one way or another, acetylcholine reached the ganglion or the denervated structures peripheral to it.

It is different in the central nervous system by whose continuous activity (according to Feldberg) acetylcholine is continuously released, even in the absence of special external stimuli. "It has, in fact, been found by all workers on the subject that, after eserine, acetylcholine appears spontaneously in the cerebrospinal fluid as well as in the venous effluent from the central nervous system."[13]

Interruption of the afferent light reflex fibers to the Westphal-Edinger nucleus creates hypersensitivity of only that group of cells which relay the light reflex normally to the iris sphincter. These hypersensitive cells continue to be under the influence of pathways from higher centers. In addition they are part of a large complex of cell groups, namely of the third nerve nucleus. Other cell groups not affected by the deafferentiating lesion lie in close proximity and are under continuous influence of their transmitter substance which is, according to Feldberg, also acetylcholine. Therefore the hypersensitive light reflex cells are under constant bombardment by acetylcholine released in the neighborhood. This explains the presence of constant sphincter spasm and miosis. As long as the cells which relay near vision impulses through the third nerve to the iris sphincter are not involved and severed from their afferent connections, the contraction to near vision is preserved.

Summary

Summarizing I may say:

1. The lesion causing the Argyll Robertson syndrome cannot be located in the iris, in the ciliary ganglion, or in the nervous connections between them. It is by no means proven that separate groups of fibers for pupillary contraction to light and pupillary contraction to near vision exist in the path between the ciliary ganglion and the iris. But even if they should exist and lesions of one group of fibers could produce dissociation of the functions as seen in the clinical Argyll Robertson syndrome, mydriasis and not miosis would have to be associated with such lesions.

2. Destruction of the ciliary ganglion renders the iris hypersensitive to acetylcholine and results in the "hypersensitive" reactions which are characteristic of Adie's syndrome.

3. Destruction of the peripheral third nerve results in mydriatic pupils which react neither to light nor to near vision.

4. Damage to or destruction of the optic

nerve results in a unilateral pupillary syndrome which resembles the Argyll Robertson syndrome inasmuch as the reaction to light is reduced or absent while the reaction to near vision is fully preserved. The syndrome may be bilateral in case of damage to the optic chiasm or one optic tract, and in this case the phenomenon may be called a "pseudo-Argyll Robertson syndrome." The syndrome is different from the true Argyll Robertson syndrome since these pupils are mydriatic in darkness, the reactions to light show low intensity and not spastic reflex shapes, and no anisocoria exists, unless complicating lesions in other neurons are present.

5. Destruction of the Westphal-Edinger nucleus frequently includes (in clinical pictures) the centers in or pathways through the third nerve nucleus for pupillary contraction to near vision. Such total destruction leads to mydriatic pupils with absence of light and near vision reactions ("absolute" mydriatic pupillary immobility). The destructive process may, however, not include the synapses or central pathways for pupillary contraction to near vision and result in unilateral or bilateral dissociation which is associated with mydriatic pupils ("mydriatic pupillary light reflex immobility").

6. There is, therefore, no reason to abandon the old doctrine that the Argyll Robertson syndrome is caused by a prenuclear lesion close to the third nerve nucleus in the last afferent neuron of the pupillary reflex arc. Deafferentiation by means of interruption of the light reflex arc would increase the sensitivity of the Westphal-Edinger nucleus and the entire pupillomotor system which depends on this nucleus. This mechanism explains the sphincter spasm and miosis. It explains the absence of the reflex to light. If deafferentiation is limited to fibers carrying impulses from stimulation by light and not from near vision, the mechanism of control for near vision is unimpaired. In this way the dissociation phenomenon of the Argyll Robertson syndrome may be produced.

However, this problem will not be definitively solved until the syndrome has been experimentally reproduced, even though all clinical and experimental facts point to the area described as the site of the lesion.

635 West 165th Street (32).

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Discussion

Dr. Carlton Melton (Dallas, Texas): I think the entire fields of ophthalmology and neurology owe thanks to Dr. Lowenstein for his excellent researches on this subject. Actually, the implications of this study go far beyond the field of ophthalmology and extent into the somewhat broader areas of neurology and physiology in general.

I find a couple of points of particular interest to me. Both of these are conceptual in nature. The first is the idea of central hypersensitivity. We ordinarily think of hypersensitivity of denervated structures as being confined to peripheral effectors, but Dr. Lowenstein postulates (and there is good evidence to support the idea) that there is parasympathetic hypersensitivity within the central nervous system. This would account for the miosis seen in the Argyll Robertson pupillary syndrome. Dr. Lowenstein's reflex shapes illustrate this very adequately.

The second point that involves something of a concept is illustrated in his differential curves of redilation following pupillary constriction to light. The two waves, showing two variations in speed with which redilation occurs, show that there is an active participation of the sympathetic system in redilation of the pupil after being stimulated by light. The implication here is that there is something of a positive feedback system involved and that redilation is not merely a relaxation of a parasympathetically induced contraction.

In Dr. Lowenstein's manuscript he pointed out that there is central differentiation, integration, and co-ordination of the accommodative and pupillary responses. However, he had no evidence as to whether or not these responses were mediated over a final common pathway.

I think some of our work might bear slightly on this subject. In working with the ciliary muscle in the excised eye preparation with the autonomic nerves intact, we found that stimulation of these retrobulbar nerves upon occasion will produce contractions of the ciliary muscle in the absence of iris movements, and will produce contraction or dilation of the iris in the absence of ciliary muscle movements. This is some slight evidence, perhaps, that these neurons, which have separate central nervous nuclei, are maintained as discrete pathways past the ciliary ganglia.

I would like to agree with Dr. Lowenstein that the final elucidation of this problem awaits the development of an experimental animal which will show the characteristic signs of the Argyll Robertson syndrome; and he has informed me that he has some very interesting leads along this line. I know everyone else joins with me in hoping that these experiments will be successful.

STUDIES ON THE AUTONOMIC INNERVATION OF THE IRIS*

JULIA T. APTER, M.D.
Chicago, Illinois

For over 50 years there has been a controversy in the published literature concerning the exact manner in which the autonomic innervation of the iris controls the size of the pupil. Recent studies suggest that the theory of reciprocal innervation of the sphincter and dilator pupillae is not tenable. The proven facts arise from three sources: anatomic, clinical, and neurophysiologic data.

The anatomic studies have demonstrated that there is an iridal constrictor muscle innervated by both sympathetic and parasympathetic nerves.[6, 20, 25, 26, 29] The iridal blood vessels have thick walls with fibers running both longitudinally and circumferentially in them.[17, 18] These are innervated by sympathetic nerves.[14, 15, 17, 18, 20, 25, 35] Contriction of these blood vessels results in dilatation of the pupil.[17] In addition, there are radially arranged smooth muscle fibers (so-called dilator pupillae) capable of contracting when an electric shock is applied to them.[1, 4, 17, 20, 30] These may be innervated by sympathetic fibers but such a connection has been demonstrated only by Cannon's law of denervation,[7] not anatomically,[18, 20]

The clinical studies add some further information. When there is a lesion of the II or III neuron of the sympathetic innervation of the iris, as in Horner's syndrome, the pupil is small but responds sluggishly to darkness and divergence, excessively to pain and fear, and not at all to the ciliospinal reflex.[15, 22] (The ciliospinal reflex was defined by Behr[5] as a small increase in the size of the pupil that occurs following a scratch on the skin of the face or neck). A lesion of the first neuron of the sympathetic gives all normal responses except that the ciliospinal reflect is absent in this case also.[5] A lesion of the third cranial nerve which supplies the parasympathetic innervation of the iris results in a large pupil immobile to all stimuli except vasoconstriction and fear.[5, 8, 18, 19, 23, 27, 32] These clinical facts are not tenable with a reciprocal innervation theory, but rather with a theory of primarily parasympathetic control of reflex changes in pupil size and a vasoconstrictor basis for drug-induced pupillodilation.

Neurophysiologic evidence heretofore has been based only on information gained by observing changes in the pupil induced by an electric shock applied to afferent nerves to the iris.[1, 4, 11, 12, 14, 16, 17, 24, 27, 29, 32]

From this work we discover that an electric shock to the dilator muscle results in pupillodilation. In addition, an electric shock to the superior cervical sympathetic ganglion produces vasoconstriction of iridal blood vessels and this results in dilatation of the pupil. These electric-shock experiments do not give evidence on the true physiology of the iris.

Still more evidence seemed to be necessary to throw light on the neurologic control of the movements of the iris. The present work aims to study the effects on pupillary motility and nerve conduction of normal stimuli only. It is based on the premise that a physiologic stimulus induces a small amount of electricity (action potential) in the nerves which transmit physiologic impulses to the responding end-organ. By this method the precise manner in which the parasympathetic and sympathetic innervation of the iris controls the size of the pupil under certain conditions was investigated.

PROCEDURE

Fifty-two cats, anesthetized by intraperitoneal Nembutal, were used in this investigation. Of these 52, 30 had the superior cervical sympathetic ganglion exposed, 12 had the ciliary ganglion exposed, and 10 had both ganglia exposed simultaneously. Four cats taken from each group had the optic nerve

* From the Manteno State Hospital.

and superior colliculus exposed as described by Apter.[3] The anesthesia was given as one dose of 42 mg. of Nembutal per kg. of body weight. This allowed the surgery to be accomplished. The animals remained quiet after surgery without more Nembutal. In 16 cats used for study of the sympathetic ganglion, the trachea was canalized to minimize the respiratory movement of the ganglion. In four a chlorided silver electrode was left in situ and the animal allowed to recover from the anesthesia before records were made of potentials from the ganglion.

The ciliary ganglion was exposed by removal of the masseter and temporal muscles, zygomatic arch, and supraorbital ridge that form the lateral wall of the orbit in cats. The eye was pushed forward and the lateral and superior recti were cut. The nerve to the inferior oblique muscle was identified and followed back to the ciliary ganglion. Chlorided silver wire or wet-thread electrodes were placed on this ganglion and pick-up was led to the Grass electroencephalograph. The stimulus was a light flashed into either eye of the cat. A hand switch governed the light and produced an artefact spike for both on- and off-stimulus. The pick-up electrodes and the light switch were led to separate channels of the electrocephalogram to eliminate artefacts that the light-switch spikes might impose on an action potential.

The superior cervical sympathetic ganglion was exposed and separated from the ganglion nodosum without cutting any detectable pre- or postganglionic nerve connections. Electrodes used were: a wet thread on the surface of the ganglion; a chlorided silver wire inserted into the ganglion; a glass insulated microelectrode; a chlorided silver wire wrapped around the ganglion and left in situ for 14 days. An electroencephalogram and a dual-beam cathode ray oscilloscope were used to pick up potentials.

Results

Action potentials typical of visual pathway activity appeared on the ipsilateral optic nerve, and brachium of the superior colliculus, and bilaterally in the pre-tectal region, and ciliary ganglion (fig. 1, 2, and 3). In all instances activity was present both when the light was turned on and when it was turned off. If this pathway was merely a constrictor pathway, it would not transmit action potentials when photic stimulation of the retina ceased. The presence of a large off-potential is indicative that this so-called constrictor pathway also controls dilatation. The findings in the ciliary ganglion are most

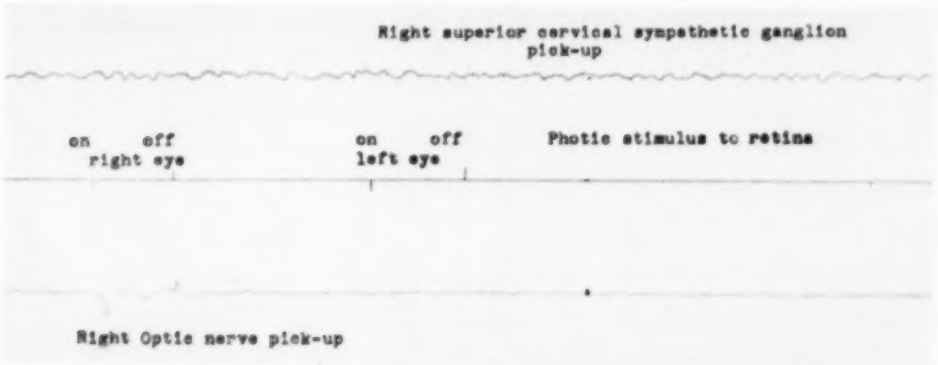

Fig. 1 (Apter). Records of action potentials from the right optic nerve and the right superior cervical sympathetic ganglion during photic stimulation of the right and the left eyes. A response to turning the light on and also to turning it off is present on the ipsilateral optic nerve. No induced potentials appear on the sympathetic ganglion.

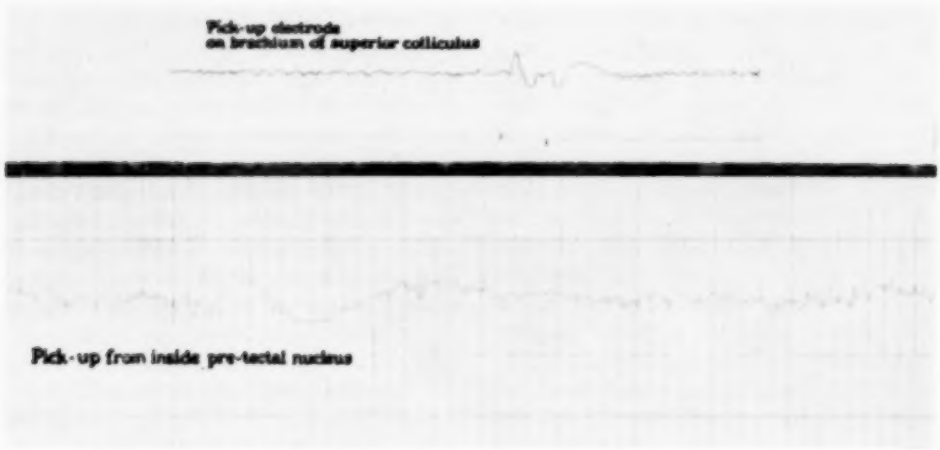

Fig. 2 (Apter). Records of potentials induced in the so-called pupilloconstrictor pathway by turning a light on and off the retina. Both on and off potentials are present in both the brachium of the superior colliculus and in the pre-tectal region.

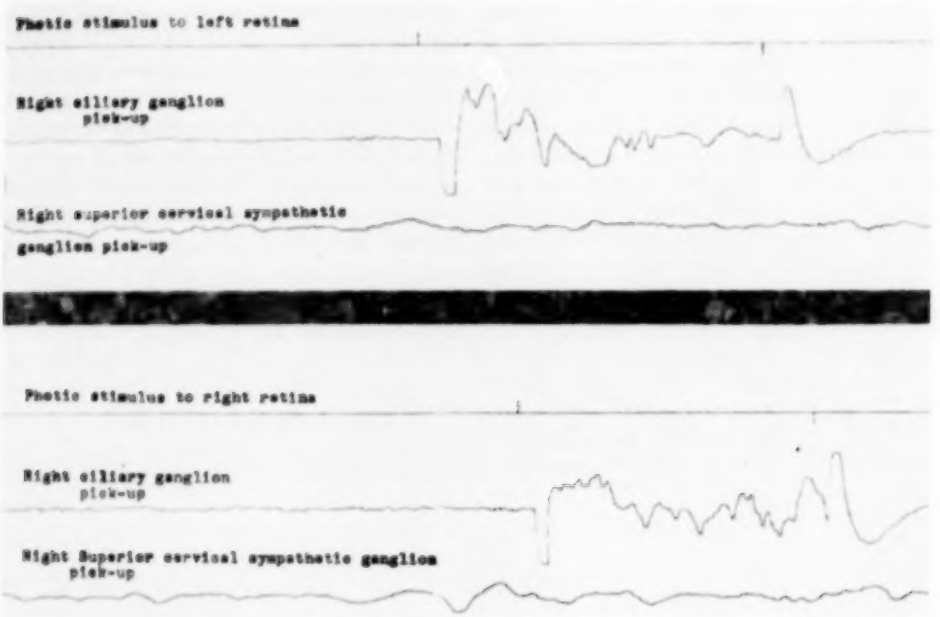

Fig. 3 (Apter). Responses in the ipsilateral and contralateral ciliary ganglion when a light is turned off and on the retina. The high potential (about 150 microvolts) following the start and the ending of photic stimulation indicates parasympathetic control of both constriction and dilatation. No responses to this stimulus appear on the superior cervical sympathetic ganglion.

notable in this regard (fig. 3). Here there was a large on-potential followed by repetitive smaller potentials which persisted throughout the duration of the photic stimulus. When the light was turned off, another larger potential appeared on the ganglion. These potentials appeared when both the ipsi- and contralateral eye were stimulated. No potentials were present during constant dim illumination.

On the other hand, a thorough search for action potentials on the superior cervical sympathetic ganglion showed no activity (figs. 1, 2, 3, and 4) associated with changes in the illumination of the retina. By means of the various electrodes used and by means of the two methods of pick-up (electroencephalograph and cathode-ray tube) all known types of potentials were sought for but not found: late and early, deep and superficial, slow and fast, ipsilateral and contralateral. Although spontaneous activity was detected by each of these means, no potentials induced by turning a light on or off the retina were present on the sympathetic ganglion. In addition, the effects of anesthesia were eliminated by leaving electrodes in situ around the sympathetic ganglion and allowing at least 24 hours to elapse before carrying out the experiment. The cats were then placed in an animal box allowing the head and neck to protrude. After the cat quieted down, the experiment was carried out during normal sleep and upon awakening. No responses followed photic stimulation of the retina (fig. 4) in either case.

It remained to be proved that the sympathetic ganglion was capable of showing changes in potential as a result of some other stimulus besides light. It is apparent from

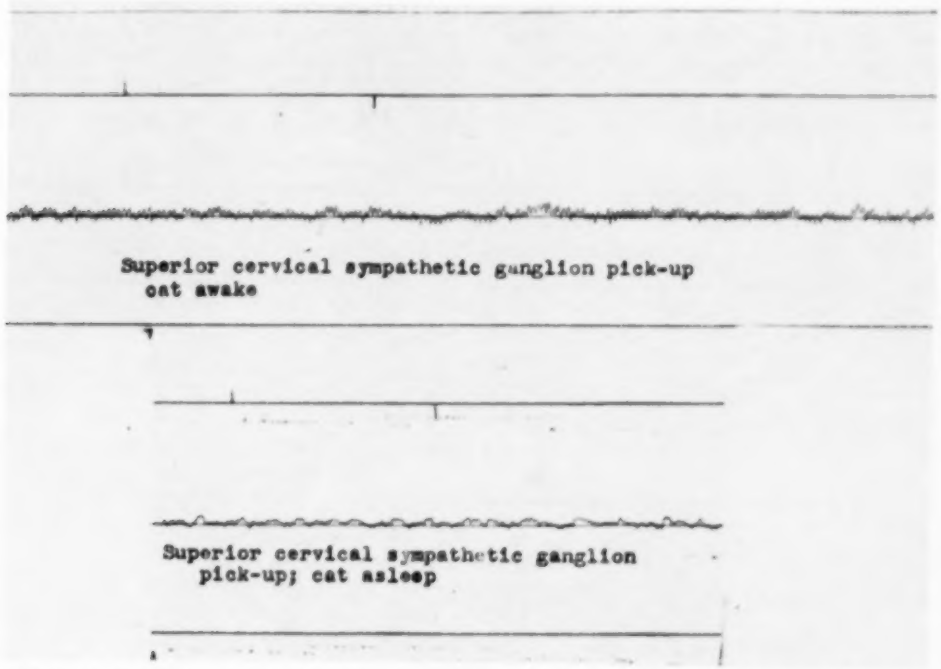

Fig. 4 (Apter). These records demonstrate that anesthesia, sleep, and awakening have no effect on the lack of response of the sympathetic innervation of the iris following photic stimulation of the retina.

Figure 4 that the spontaneous activity during sleep is of lower amplitude and lower frequency than upon awakening. This finding merited further study although no light-induced potentials were present either during sleep or while the cat was awake. If the cat is asleep, but not anesthetized, a pinch on the ear or a loud noise will awaken the animal. This arousal is indicated by an instantaneous dilatation of the pupil.

Simultaneous with this dilatation the increase in frequency of the spontaneous ganglionic activity appears. It resumes a lower frequency when the cat again falls asleep and the pupil constricts (fig. 5). This activity resembles the "arousal potentials" in the thalamus of cats, found by Snider. It is not specific for dilation of the pupil but rather is associated with the general sleep mechanism. It does indicate, however, to what extent the superior cervical sympathetic ganglion is capable of showing changes in potential. Therefore, the absence of changes in potential associated with dilatation due to darkness must mean an absence of activity in the ganglion during that dilatation. There were no potentials induced by electrical stimulation of peripheral nerves or by a pinch on the ear or by other methods of exciting the animal.

What, then, was the meaning of the experiments performed by Langley and Anderson[17] which seemed to prove sympathetic antagonism to parasympathetic action? They applied a galvanic shock to afferents of the superior cervical sympathetic ganglion, and the pupil dilated. It appears that Langley's experiment demonstrated that sympathetic nerve fibers reach the iris from the superior cervical sympathetic ganglion; that an electric shock anywhere on the iris dilates the pupil, as did Kuntz and Richins.[16] He did not, however, throw any light on the true function of the sympathetic innervation of the iris.

Conclusions

For half a century a hypothesis of reciprocal innervation of the iris by sympathetic and parasympathetic nerves with resultant antagonistic actions on the pupil has held sway. The data of the present experiments

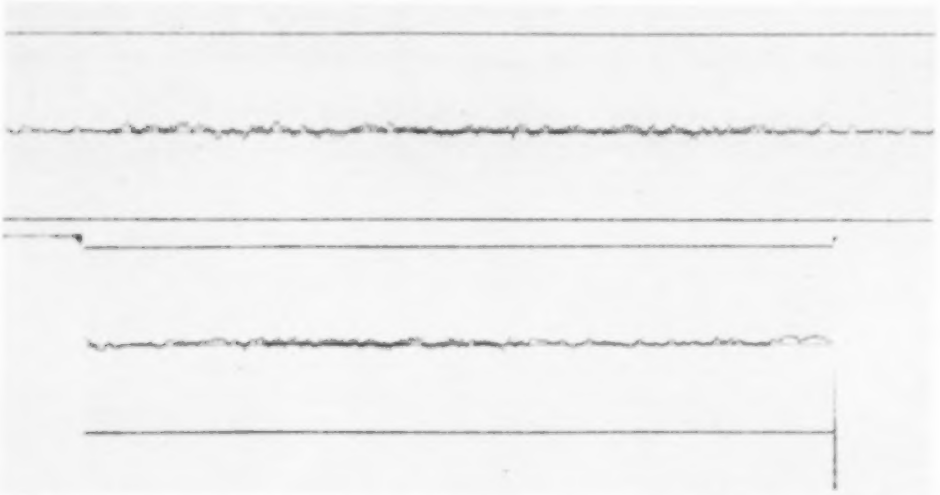

Fig. 5 (Apter). This record shows changes in spontaneous activity induced in the superior cervical sympathetic ganglion by arousal of a sleeping cat. When the cat awakes, his pupil dilates. This dilatation is indicated by the faint line drawn by an assistant. There is close correlation between this arousal dilatation and increased frequency and amplitude of spontaneous activity.

coupled with most previous anatomic, clinical, and neurophysiologic evidence require that the hypothesis be discarded. Instead, we must conclude that the movements of the iris are controlled by two independent variables. One is the parasympathetic innervation which varies swiftly, through wide limits. The other is the sympathetic innervation which varies slowly through narrow limits The important reflexes such as those to convergence, divergence, light, and darkness are part of parasympathetic control. The lesser reflexes such as the ciliospinal reflex may be mediated solely by the sympathetic. On the other hand, the sympathetic probably acts constantly with slow, small variations to control the resting size of the pupil: to prevent extreme miosis during maximal action of the parasympathetic action on the sphincter.

Superimposed on this innervational control is the control imposed by changes in the caliber of iridal blood vessels. Vasoconstriction induced either by nervous, hormonal, or drug action results in dilatation whether the parasympathetics are active or inhibitive.[8-10, 15, 18, 31, 33-35] The sympathetic control of the iris dilator muscle is incapable of truly opposing parasympathetic action. The case in point is that the ciliospinal reflex dilatation cannot be elicited in bright illumination.[5]

No study on the pupil is complete without an analysis of new facts in the light of pupillographic studies by Lowenstein and his co-workers.[21-23] They have examined the effects of numerous nervous defects on pupillary activity. They seem to assume that the dilator muscle alone is innervated by the sympathetic nerves. This is, of course, inconsistent with the facts. Their work is indisputable insofar as performance of the pupil is concerned. However, a re-evaluation of the pathogenesis of changes in this performance should be done on the basis that the sympathetics innervate the constrictor muscle and the blood vessels as well as the dilator muscle. When this is done, their results confirm the findings and conclusions of the present study.

Summary

1. The extent to which the parasympathetic and sympathetic innervations of the iris participate in the control of the size of the pupil is studied by means of action potentials on the ciliary ganglion and on the superior cervical sympathetic ganglion.

2. The ciliary ganglion shows marked action potentials during both the light and the darkness response. The superior cervical sympathetic ganglion is inactive during these responses.

3. The sympathetic ganglion shows electrical activity indicative of changes in tone during arousal from sleep, associated with the awakening dilatation of the pupils.

4. The significance of these data coupled with previous observations by other workers is analyzed. It seems most logical to suppose that the sympathetic and parasympathetic innervations of the iris are independent variables: the sympathetic varying slowly through narrow limits and the parasympathetic varying swiftly through wide limits. Superimposed on this activity are the variations in pupil size imposed by vasoconstriction of iridal blood vessels.

7316 South Paxton Avenue (49).

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Discussion

Dr. Hunter H. Romaine (New York): My congratulations to Dr. Apter not only for a fine contribution to a better understanding of the neurophysiology of the iris, but for an experiment remarkably well devised and executed. It is very true that contention has existed in the relation between the sympathetic and parasympathetic systems for many years. The concept of a strict balance between the two systems has proved untenable in many physiologic theories. This seems to hold true in the iris. The mechanism is obviously much more complicated than was anticipated when the first observations were made. The greatest difficulty in the present methods of study would seem to lie in the

interpretations of the electrical potentials in the analysis of various nervous stimuli. I am puzzled by the interpretation of the "increased activity" in Figure 5. The wave is suspiciously like the 60-cycle interference which is present before and after the dilatation. Someone touching the animal during the waking process might cause such interference.

In the Department of Electrophysiology of the Research Department at the New York Eye and Ear Infirmary recent attempts to study electrical potentials of the ciliary body have been complicated by difficulties in the interpretation of these electrical potentials. Further studies using this approach may be helpful in determining the veracity of present theories on ciliary activity. Any clarification of the issues, as has been brought forth in this paper, is a fine contribution to medical science.

DR. OTTO LOWENSTEIN (New York): While listening to Dr. Apter's well-presented paper, I was puzzled for some time by her results. It was only at a rather late point in her paper, when she described that sympathetic activity could not be recorded while the pupil dilated "except for the dilatation at the moment of awakening," that it became clear to me where to look for the solution to the enigma. I should like to explain this briefly.

Pupillary dilatation, which occurs reflexly when sensory nerves such as the sciatic nerve are stimulated, is produced by at least three basic mechanisms: (1) contraction of the dilatator muscle in response to sympathetic impulses; (2) inhibition of the third nerve nucleus; (3) neurohumoral mechanisms. When the sympathetic is cut, thus eliminating the first mechanism, the pupil is still able to dilate though this dilatation, pupillographic records indicate, is slow and incomplete. When the pupil is parasympathectomized and the second (inhibition) mechanism is eliminated, the mydriatic pupil of the awake animal dilates fast and completely to psychosensory stimuli. Reflex dilatation is abolished only when both of these mechanisms are eliminated.

When a sensory nerve, for example the sciatic nerve, is strongly stimulated in a cat under general Nembutal anesthesia, the pupils dilate slowly and become submaximal. The dilatation movement shows pupillographically all characteristics of parasympathetic inhibition in which sympathetic activity has no part. It can be easily shown that under the condition of anesthesia, sympathetic activity is really absent, because (1) both pupils dilatate equally, at the same speed and to the same extent, when the cat is unilaterally sympathectomized, and (2) pupillary dilatation is abolished on the parasympathectomized side when the third nerve is cut, or the ciliary ganglion removed, or atropine instilled into one conjunctival sac. It is, therefore, easily explained that Dr. Apter could not record any sympathetic activity in response to sensory stimuli because there was none as long as the animal was in general anesthesia. She had produced dilatation by parasympathetic inhibition over the well known ascending pathways in the reticular formation. But at the moment when the animal awoke, sympathetic activity came into play and her records showed it.

Dr. Apter reports that she reviewed the literature of the last 50 years and came to the conclusion that the sympathetic nerves to the iris change the size of the pupil primarily by changing the caliber of iridal blood vessels, while the dilator muscle acts passively to allow maximal dilatation and prevent maximal constriction as a result of slow tonic impulses supplied by the sympathetic nerves.

These theories are in part very old and go back to the 16th century when Argenterius (1556), Fabricius (1600), Méry (1704), and Ferrein (1741) taught that pupillary contraction was caused by an influx into the iris blood vessels, thereby causing the iris to swell (erectile theory). Dilatation was supposed to be caused by shrinkage of the iris due to decrease of influx. This theory, more or less modified, found followers (Sömmering 1788, Döllinger 1817, Gaddi 1845) in each subsequent century, but scarcely adherents to the entire theory in our century (Vennemann 1903 and Samaja 1926 agreed in part) except for Langworthy and Ortega who, in 1943, tried to revive the old theory. A dilatator muscle had been described in the iris stroma since 1837 (Lauth, Valentin, Schwann, and at least 40 other authors), and in Bruch's membrane since 1864 (by Henle and successively by at least 100 other authors). Nevertheless, there were still a number of authors who did not, or not fully, believe in its existence or its function as a dilatator. They adopted a modified vascular theory, the so-called vascular contraction theory (Hildebrand 1785, Troxler 1803, Kluge 1828, Gruenhagen 1870; in part, Koganei in 1885 and, again, Langworthy and Ortega in our century, 1943).

There is much evidence against the correctness of the vascular theories; only a small part of it shall be mentioned:

1. Pupillary dilatation and vascular constriction have each their separate nerve supply which can be individually stimulated and cut; this can be done either at the level at which the thoracic roots emerge from the spinal cord (Claude Bernard 1862, Langley 1897), at the ansa Vieussenii (François-Franck 1878), the cervical sympathetic chain (Schiff 1872, Littauer 1892), or at various sites of the diencephalon (v. Sallmann and Lowenstein 1955).

2. Pupillary dilatation fibers and vascular fibers can be separated by threshold stimulation (v. Arlt 1869, Schiff 1872, Langley and Anderson 1892, Gruber 1915, Eccles 1935). For each group of nerve fibers which are separable by threshold stimulation, separate components of the action potential pattern can be recorded (Bishop and Heinbecker 1932).

3. Pupillary dilatation precedes vascular events; therefore the former cannot be caused by the latter. This was first pointed out as early as 1604 by Laurentius and stressed since then by numerous investigators.

4. Nerve stimulation after death either at the scleral limbus or in the cervical sympathetic results in dilatation; this was frequently shown between 1846 and 1895 (Ruete 1846, Weber 1846, Brown-

Séquard 1849, Budge and Waller 1851, Budge 1852, Donders 1864, Schiff 1867, François-Franck 1878, Langendorff 1891, Reid 1895). Vascular occlusion did not prevent pupillary dilatation upon stimulation of the cervical sympathetic, although the entire blood supply to the head was eliminated (Schoeler 1869, Hensen and Völckers 1879, Shiff 1896, Berteau and Jones 1950). Stimulation of the vagus causes the blood pressure to drop, but does not affect the pupil (Langley and Anderson 1892).

5. Finally, it should not be forgotton that the pupils always dilate under the influence of emotions, whether a man blushes or turns pale.

The controversies concerning the existence of the dilatator muscle, as well as the modes of its actions, belong mainly to the 18th and 19th and not to the 20th century. The best physiologists and anatomists of their time had participated in those polemics which were filled with dramatic and even tragic moments, as for instance the one when Gruenhagen (1892-93) after 30 years of experimental research and literary fights finally became convinced by the classical work of Langley and Anderson (1892) of the existence of a dilatator muscle he had emphatically denied.

It is not in the interest of scientific progress to resume those three-century-old fights* which were concluded because they had exhausted their arguments.

I am sorry to be so negative, Dr. Apter, but unfortunately I have no choice.

DR. JULIA T. APTER (closing): May we have the last slide again, please? Dr. Romaine was kind enough to tell me what his discussion was going to cover, and therefore I had time to prepare a slide to show you what 60-cycle interference does look like. It does not resemble the arousal potentials which were present in my unanesthetized cats.

I do think it was very kind of Dr. Romaine to be so generous with his remarks, and I am only hoping that in his department (and in others, too) there will be more work done on this subject, because I do feel that if in unanesthetized cats we do get action potentials showing that the cat is awake but nothing associated with photic stimulation of the retina, then we should not consider the sympathetic is acting during dilatation due to darkness.

[Slide] As far as some of Dr. Lowenstein's questions are concerned, I did not mean to say that the blood vessels do all the work during photic stimulation of the retina. The sympathetics do not work during dilatation of the pupil due to the absence of light.

These are the 60-cycle findings. You can see how different they are from the arousal potentials in the previous slide. In the case of the blood vessels, these constrict under the action of drugs, and it is precisely in that connection that I want to urge that the actions of drugs be considered carefully before it is decided that they dilate the pupil by acting on the dilator muscle. They dilate the pupil by acting on the blood vessels as does circulating adrenalin. If you will consider, I think you will see that most pupillodilating drugs are vasoconstrictors except those that paralyze the action of the parasymathetics. Experiments with electrical stimulation induce artificial results that throw no light on physiologic activity. All of the data which seem to support the antagonism theory are derived from electrical stimulation of the sympathetic nerves and are not as reliable as data derived from physiologic stimuli.

* An experimental-historical review of these problems was prepared in our laboratory and is being published under the title: "The mechanisms of pupillary dilatation."

SOME MECHANISMS OF CENTRALLY INDUCED EYE PRESSURE RESPONSES*

LUDWIG VON SALLMANN, M.D., FRANK J. MACRI, PH.D., THEODOR WANKO, M.D., AND PATRICIA A. GRIMES, B.A.
Bethesda, Maryland

Discussions of central nervous influences on the intraocular pressure played an essential part in early theories of glaucoma. It was only in the last few years, however, that the problem was studied on an experimental basis.[1-3] It was shown in these investigations that electric stimulation in certain areas of the interbrain of the cat caused acute rises and falls of the intraocular pressure which either paralleled alterations in the systemic blood pressure and other autonomic functions, or were dissociated from them.[3]

Although attempts were made to relate the shape of the pressure tracings to the nature of the stimulation effect, there remained some doubt as to whether the described responses of the intraocular pressure arose

* From the Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare.

from variations of intraocular blood flow or from contraction of extraocular smooth or striated muscles. In the present work, several approaches were utilized to establish the mechanisms by which such centrally elicited changes of the intraocular pressure were brought about. The analysis was limited to the effects of stimuli in the posterior ventral hypothalamus, since from this area typical response patterns of generalized sympathetic activity can be reproduced with great regularity. Only changes occurring during relatively short stimulation periods were considered. Thus, the study was not concerned with central nervous influences on aqueous dynamics.

Materials and Methods

Observations were made and evaluated on 42 adult cats weighing between 2.0 and 3.5 kg. Chloralose (50 mg./kg., intraperitoneally) served as an anesthetic in 21 instances. In other experiments anesthesia was induced with intravenous sodium pentothal (16 cats) or with ether (five cats). As in previous work preparatory trephine craniotomy preceded the stimulation experiment by four to seven days. On this occasion, the superior cervical ganglion on one side was removed in 10 cats; in several preparations, the nictitating membranes were excised at the same time to facilitate the observation of pupillary reactions. After several days, clinical examination and pharmacologic testing demonstrated the completeness of ganglionectomy.

All animals were tracheotomized to be prepared for artificial respiration when needed. As a rule both common carotid arteries, the vagi, and the preganglionic cervical sympathetic nerves were isolated in the trigonum caroticum and thread loops were placed around them. The left external jugular vein and left femoral artery were cannulated for continuous pressure recordings, whereas, a cannula in the right femoral vein allowed for the administration of drugs.

For temperature measurements, the sclera was exposed by incision through the conjunctiva and Tenon's capsule in the temporal upper or lower quadrant of the globe. The sclera was incised, as in a cyclodialysis operation, at a distance of four to six mm. from the limbus, the perichoroidal space in this area probed cautiously with a fine spatula, and the wound temporarily closed with a 5-0 silk suture.

The animal thus prepared was placed on a modified Horsley-Clarke stereotaxic apparatus. With the head fixed in the usual manner, the external auditory canals and the inferior orbital margins defined the horizontal reference plane.

After heparinization of the animal, the cannulas in the various vessels were connected to Sanborn electromanometers; 27-gauge hypodermic needles attached to either rigid, self-supporting lead tubing, or to fine polyethylene tubing were inserted in the anterior chamber of each eye. All connector tubings contained a solution of 0.9-percent sodium chloride, and fed into the recording systems. Careful calibration of the instrument anteceded the actual recording of the various pressures. In later experiments, Statham transducers were conveniently combined with Sanborn carrier preamplifiers for intraocular pressure recording.

For thermometry in the potential space between ciliary body and sclera, a fine silver-beaded 35-gauge alumel-chromel thermocouple was introduced through the scleral wound and fixed in situ with pre-placed sutures. Continuous temperature tracings were obtained either on a Speedomax instrument (Leeds and Northrup) or on a Sanborn recorder with a low-level (chopper) preamplifier. With these two instruments temperature differences of 0.05 and 0.2°C. respectively could be measured. An Esterline Angus milliameter, used in conjunction with the Sanborn instrument, further increased the sensitivity of this system; this was achieved by connecting the milliameter to the galvanometer of the recorder and interposing a variable resistor between these

two parts. With the resistor, the sensitivity could be regulated within limits. Such an arrangement provided for tracing of temperature changes in the order of 0.005°C.

A slight modification of the Horsley-Clarke stereotaxic apparatus employed in a previous study[4] facilitated access to the ventral region of the neck for the manipulation of vessels and nerves. With the animal's head fixed as described, the electrodes were introduced into the left hemisphere in a paramedian vertical plane 2 mm. from the midline, at co-ordinate settings from 8.0 to 12 mm. in front of and from 8.0 to 5.0 mm. above the interaural line.*

Four-pronged bipolar electrodes were made of fine entomologic pins, 0.4 mm. in diameter, re-enforced at their base by hypodermic needle tubes, and were set 1.0 mm. apart. Two-pronged electrodes, which consisted of stainless steel 24-gauge hypodermic needle stock, were 2.0 mm. apart. Wire connections of the individual needle electrodes led to the output of a switch box, which permitted the change of the polarity of the selected pair of terminals. The electrodes were insulated with Tygon; their tips were bevelled to sharp points and freed of insulating material for a distance of 0.5 or 1.0 mm.

Square wave stimuli delivered by a Grass, S-4, stimulator were applied throughout these experiments at a frequency of 60 cycles per second and a duration of two milliseconds. Voltages varied between two and eight volts. Tissue resistance between the electrode needles, 1.0 mm. from tip to tip, ranged between 50,000 and 70,000 ohms.

Terminal vessels at the limbus were observed through a Bausch and Lomb stereomicroscope or a Zeiss otoscope. Pupillary movements were noted visually and graded in all experiments. Movements of the nictitating membrane or of the lids and globe were also grossly observed.

* The Atlas of Jasper and Ajmone-Marsan[5] was used in determining the Horsley-Clarke co-ordinates for desired placing of the electrode tips.

The role played by centrally produced systemic blood pressure rises in eye pressure responses was studied in four experiments with a technique devised for regulating the blood flow to the head of the cat preparation. With the anesthetized animal under artificial respiration, the chest was opened and the vertebral arteries ligated at their origin. The heavy stem of a Y-shaped polyethylene cannula was then introduced into the abdominal aorta above its bifurcation. Finer polyethylene tubings, forming the upper part of the "Y," were inserted cephalad into the ligated common carotid arteries. The blood flow to the head was adjusted to desired pressure levels by means of a screw clamp on the tube. For recording aortic blood pressure and the carotid blood pressure respectively 24-gauge hypodermic needles were inserted into the tubing between abdominal aorta and clamp and between clamp and carotids and connected to electromanometers. By the appropriate setting of the clamp, the effective head blood pressure could be lowered to any level below that of the aortic blood pressure.

Results

General effects

Electric stimuli applied in the posterior ventral hypothalamus usually caused—in addition to the known elevations of intraocular pressure and blood pressure, pupillary dilation, and retraction of the nictitating membrane—synchronous increase of venous blood pressure and a rise of temperature in the anterior subscleral space (fig. 1).

The time lag between onset of stimulus and onset of individual responses could not be measured accurately with the presently applied technique, but was brief (less than one second). However, with the four-channel recording instrument the time interval between stimulus onset and response maximum could be estimated. The analysis of many records indicated that maxima of blood-pressure rises were reached one to two seconds before the eye pressure had ascended to peak levels. Measurable temperature changes

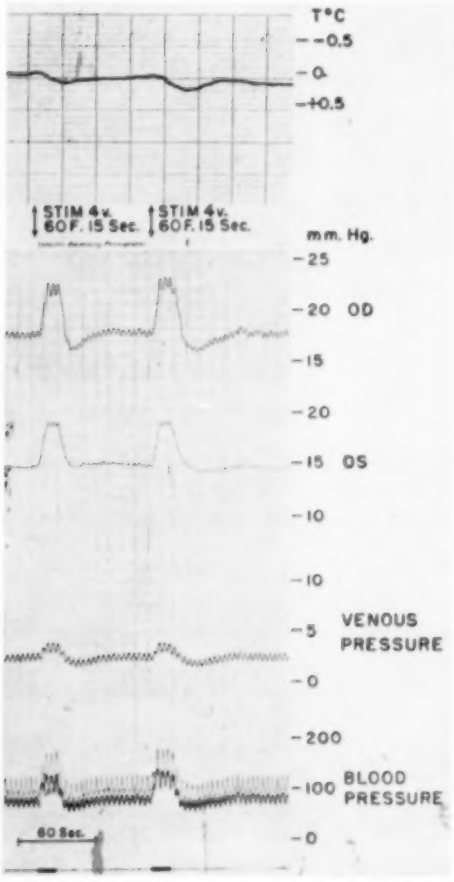


Fig. 1 (von Sallmann, et al.). *Associated responses to central stimulation of blood pressure, venous pressure, intraocular pressure and subscleral temperature.*

Pupils responded fast and maximally during stimulation. Nictitating membrane removed. Temperature was measured in O.D. Position of positive electrode: 12 mm. anterior to and eight mm. above the interaural line. Pentothal anesthesia.

In all illustrations O.D. and O.S. refer to the intraocular pressure of the right and the left eye respectively.

showed delay of onset in a range of one to 10 seconds, and lasted longer than the responses of the other autonomic functions. Stimulation-induced reactions of conjunctival and superficial episcleral vessels around the limbus were either absent or minimal, transient, and erratic. Certainly no parallelism was noted between eye and blood pressure variations and caliber changes of the pericorneal vessels.

The amplitudes of pressure changes rose with stronger stimuli. Elevations of the eye pressure reached values up to 9.0 mm., those of the arterial blod pressure measured maximally 125 mm., and of the venous blood pressure, on one occasion, 9.0 mm. Hg. These marked responses were associated with increases in the subscleral temperature of about 1.0°C. or less, depending on the magnitude of pressure responses.

The tracings of both eyes showed an ascent of the curve to approximately the same height, even when the pre-stimulation pressure levels differed by several millimeters. Previous observations[3] on the continuance of centrally induced eye pressure changes were confirmed: peak pressure levels were either maintained and fell abruptly to original values at cessation of the stimulus, or returned gradually to the base pressure at various time intervals during stimulation. The first type of pressure responses occasionally displayed additional increments of rise about 20 seconds after onset of a prolonged stimulus, accompanied by a slow augmentation of the mydriatic effect.

In several experiments on animals under chloralose anesthesia, centrally evoked eye and blood pressure changes, pupillary dilation, and retraction of nictitating membranes were compared with respect to stimulus strength. The duration of stimulus was limited to 15 seconds to avoid injury to the tissue by repeated stimulation at the same point. The observed reactions do not represent, therefore, maximal effects in all instances. Table 1 lists in one example the thresholds of effective stimuli and also indicates the type of reaction. Two-volt stimuli in this preparation gave rise to measurable increases of eye and blood pressure and a slow, slight pupillary dilation. The nictitating membrane did not retract, even when the strength was increased to three volts, in contrast to the marked effects of this stimulus

TABLE 1

COMPARISON OF RESPONSES OF INTRAOCULAR PRESSURE, BLOOD PRESSURE, PUPILS AND NICTITATING MEMBRANE TO CENTRAL STIMULI OF VARYING STRENGTH

Voltage	Intraocular Pressure Change mm. Hg (O. S.)	Blood Pressure Change mm. Hg	Pupillary Dilation	Nictitating Membrane Retraction
1	None	None	None	None
2	0.5	10.0	Slow, slight	None
3	2.8	30.0	Fast, medium	None
4	5.3	45.0	Very fast, max.	Fast, partial
5	7.0	63.0	Very fast, max.	Fast, complete
6	8.5	77.5	Very fast, max.	Fast, complete
6	8.3	77.5	Very fast, max.	Fast, complete
Responses after sectioning of left cervical sympathetic nerve:				
6	8.8	72.5	Very fast, max.	None

Electrode tip was placed two mm. left of midline, seven mm. above and 11 mm. in front of the interaural line. Duration of stimulus—15 seconds.

on the other functions. Five-volt stimuli produced maximal reactions in all four effector systems. Sectioning of the cervical sympathetic nerve at the six-volt stimulation level neither influenced perceptively the eye and blood pressure changes nor the extent and speed of the pupillary dilation, but did abolish the response of the nictitating membrane. A 1:10 ratio of eye to blood pressure rises was approximately maintained at the various stimulation levels.

The slopes of the eye and blood pressure increments varied as a function of stimulation strength. That is, in general a stronger stimulus induced a faster rise. A detailed study of this recruiting phenomenon is forthcoming.

The character of the intraocular pressure response to hypothalamic stimulation appeared to be independent of the three employed anesthetic agents. However, stimulation thresholds at which generalized sympathetic activity could be evoked were commonly lower in cats under chloralose anesthesia (1-4V), as compared with pentothal or ether anesthesia (4-8V). Spontaneous and rhythmic rolling movements of the eye sometimes interfered with the recording of the intraocular pressure changes in the various forms of anesthesia. This disturbing condition was alleviated or abolished by supplemental anesthesia. In chloralose-anesthetized animals, small doses of sodium pentobarbital (1.0 to 3.0 mg./kg. intravenous) were sufficient for this purpose.

EXTRAOCULAR MUSCLE EFFECTS

1. *Smooth muscles.* In previous work it was tentatively concluded that the sudden rise of intraocular pressure upon stimulation in the posterior ventral hypothalamus originated in part from the contraction of the smooth orbital muscles. The concomitant widening of the lid fissures and retraction of the nictitating membrane suggested this mechanism. Indeed, electric stimulation of the cervical sympathetic trunk elicits this triad of responses: contraction of smooth muscles of the orbit, pupillary dilation, and, not infrequently, increase of intraocular pressure. Since it is generally held that this nerve represents the only pathway for transmission of sympathetic impulses to the orbit, two series of experiments were conducted to possibly eliminate smooth muscle effects on the intraocular pressure.

In a group of 10 experiments, the cervical sympathetic nerve was sectioned preganglionically, on one side, either prior to electric stimulation (fig. 2) or during the period of sustained centrally elicited intraocular pressure rise (fig. 3). Neither of the two procedures influenced the extent of the eye pressure response, which resembled closely

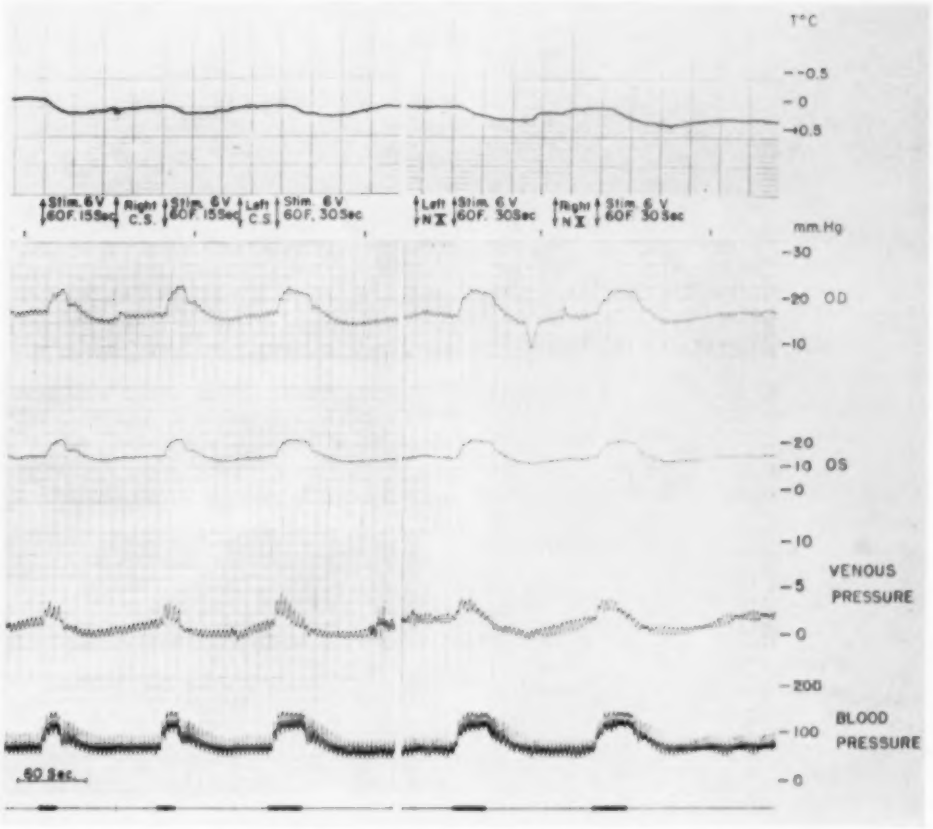


Fig. 2 (von Sallmann, et al.). *Influence of sectioning the cervical sympathetic and vagus nerves prior to stimulation.*

Severing of nervous pathways had no effect on the responses of intraocular pressure, temperature, and blood pressure. Pupillary dilation remained fast maximal during each stimulus. Nictitating membrane removed. Temperature was measured in O.D. (CS) Section of cervical sympathetic nerve. (NX) Section of vagus nerve. Position of positive electrode: 12 mm. anterior to and six mm. above interaural line. Pentothal anesthesia.

previous stimulation effects on this eye and pressure changes produced by the same stimulus on the contralateral eye (table 1). Subsequent severing of the other cervical sympathetic nerve also did not influence the response pattern (fig. 2). Retraction of the nictitating membrane under these conditions was abolished. However, after approximately 20 seconds of continued stimulation a slow incomplete retraction was observed.

In another group of 10 animals, excision of the superior cervical ganglion on one side preceded the stimulation procedure by four to seven days. A Horner-like syndrome developed in the following days and the pupillary dilation, which resulted from intravenous injection of adrenalin in a dose of 0.1 μg./kg., indicated sensitization of the effector organ. The eye pressure of the operated side was, on an average, 2.0 mm. lower than that of the control eye. As in the animals with unilaterally sectioned cervical sympathetic nerve, no differences of the centrally elicited eye pressure changes were apparent

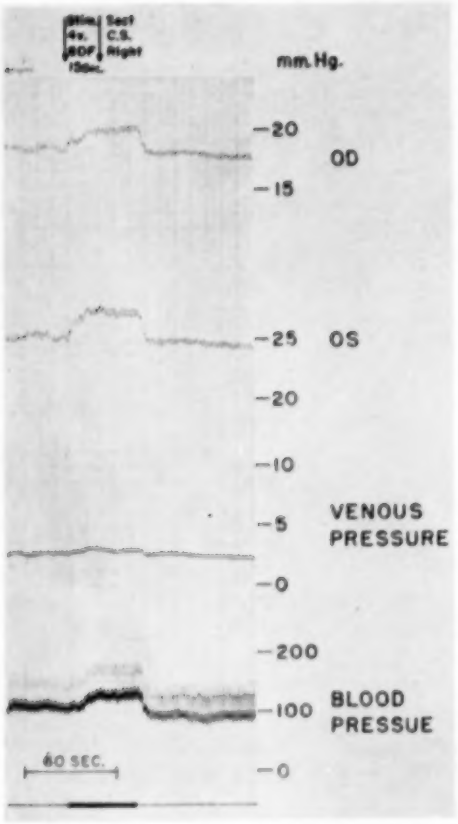


Fig. 3 (von Sallmann, et al.). *Influence of sectioning the right cervical sympathetic nerve during stimulation.*

Response patterns, after sectioning, remained unchanged including the submaximal pupillary dilation. Nictitating membrane removed. Cervical sympathetic nerve was sectioned 15 sec. after the onset of a 45-sec. stimulus. Position of positive electrode: 12 mm. anterior to and six mm. above interaural line. Pentothal anesthesia.

between the denervated and the intact eye (fig. 4). Unilateral or bilateral interruption of the sympathetic pathway did not alter the usual fast and nearly maximal dilation of pupils produced by stimulation in the ventral posterior hypothalamus.

Vagotomy was performed in six experiments on one or both sides after the results of sectioning the cervical sympathetic nerves had been studied. The effects of hypothalamic stimulations were not modified by these procedures.

2. *Striate muscles.* In view of the topographic relationship of the selected stimulation area in the hypothalamus to oculomotor pathways and nuclei, it was unlikely that striate muscle activity interfered with intraocular pressure changes in the present experiments. Nevertheless, to preclude this possibility, Decamethonium Bromide was administered intravenously (0.2 mg./kg.) in nine experiments. Rotation and retraction of the globes, propulsion of the nictitating membrane, and lid spasm ensued almost immediately. The intraocular pressure rose steeply in a smooth ascent for 20 mm. Hg (fig. 5) and very gradually declined to the base pressure within 30 to 60 minutes, in

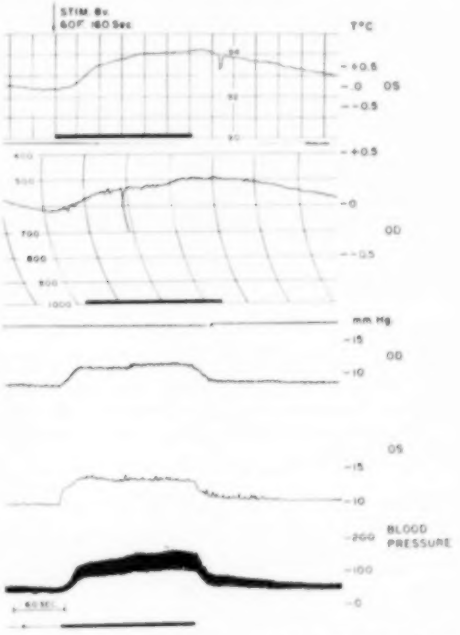


Fig. 4 (von Sallmann, et al.). *Influence of superior cervical ganglionectomy.*

The ganglionectomy was performed on the right side, seven days prior to the experiment. Response patterns were similar in both eyes. Pupils O.U. dilated fast and submaximally. Nictitating membrane removed. Position of positive electrode: 10 mm. anterior and eight mm. above interaural line. Chloralose anesthesia.

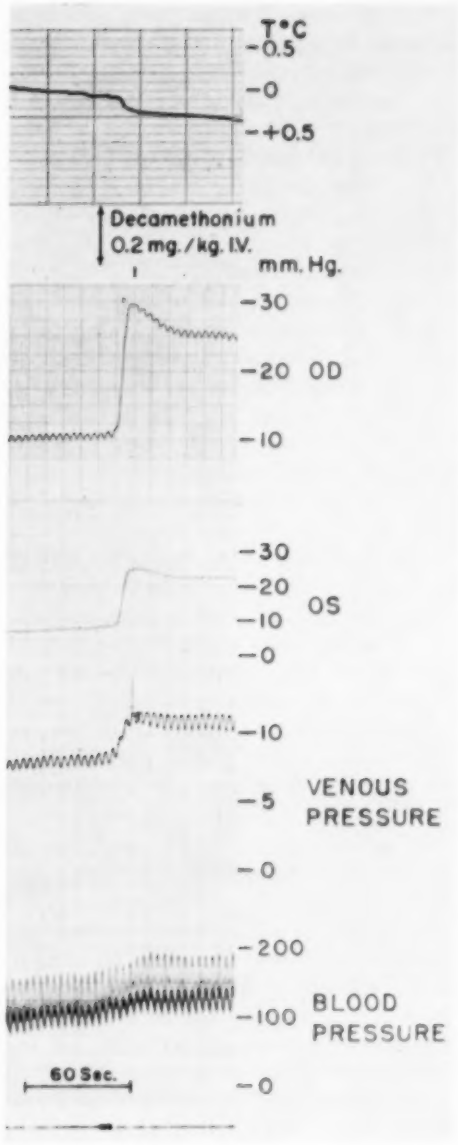


Fig. 5 (von Sallmann, et al.). *Effect of Decamethonium.*

A rapid rise of high amplitude of intraocular pressure was accompanied by a temperature increase and a moderate blood pressure elevation. The latter was not observed in other experiments. A marked drug-induced propulsion of the nictitating membrane prevented observation of the pupils. Pentothal anesthesia.

contrast to the short-lived fasciculation seen on other skeletal muscles. Successively injected doses of the methonium compound regularly produced eye pressure rises of smaller amplitude and shorter duration. In one experiment, a concentric needle electrode was placed in the belly of the lateral rectus muscle of one eye after resection of the lateral orbital wall, to record muscle activity on the cathode ray oscilloscope. Two brief bursts of discharges shortly after the injection of the pharmacologic agent accompanied the early stage of muscle contracture and were followed by electrical silence (fig. 6). Stimulation of the hypothalamus, at a time when general neuromuscular junction blockade was still evident but premedication eye pressure levels were almost restored, produced pressure changes similar to those observed before the injection of decamethonium.

Circulatory effects

Relative synchrony and similarity of centrally elicited blood and eye pressure responses suggested that changes of the intraocular pressure reflected mainly variations in the general blood pressure. It was not clear, however, to what extent local vascular events participated in the response pattern. Biomicroscopic observations on the pericorneal vessels and temperature measurements in an area near to a dense intraocular vascular bed provided some information on this point.

As mentioned before, examination of the limbus vessels with × 40 magnification in green light did not disclose marked or consistent responses in this vascular bed during stimulation. Vasodilation or constriction was either questionable or slight, and of a fleeting nature. These minimal changes seemed to be independent of concomitant blood and eye pressure reactions to central stimulation.*

* Electric stimulation of the cervical sympathetic nerve also did not effect the perilimbal vessels under the selected experimental conditions.

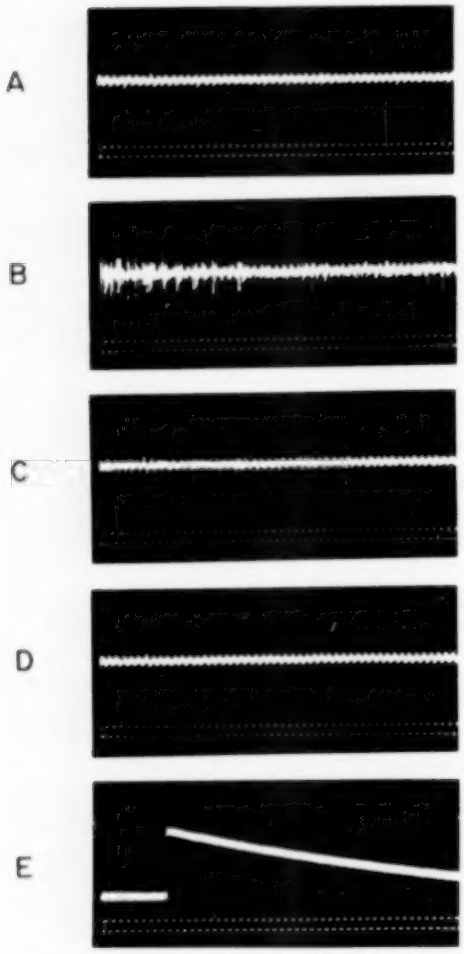


Fig. 6 (von Sallmann, et al.). *The electromyogram of the lateral rectus muscle after the administration of Decamethonium bromide.*

(A) Before Decamethonium. (B) Six sec. after Decamethonium (0.2 mg./kg.; intravenous). (C) 15 sec. after (B). (D) 54 sec. after (B). (E) Calibration 0.1 mV, 60 c.p.s. (Electromyogram was kindly prepared by Dr. Hans Bornschein.)

In contrast to these results, temperature measurements in the subscleral space gave some insight into the behavior of intraocular vessels during stimulation in the posterior ventral hypothalamus. Rises of temperature ranging between 0.03°C. and 1.0°C. accompanied, regularly, elevations of eye and blood pressure. A quantitative correlation between temperature and pressure changes could not be established, possibly because the thermocouple beads were not placed at identical sites and the trauma of the introduction of the thermocouple may have varied from experiment to experiment. It appeared, then, from these experiments that the eye pressure variations were closely linked to intraocular blood flow.

The common carotid arteries were occluded unilaterally and bilaterally during stimulation to further clarify the relationship between blood flow and eye pressure. Ligation of one carotid artery, at a time when peak levels of the centrally elicited pressure responses were maintained, produced sharp pressure falls limited to the ipsilateral eye (fig. 7).

The amplitude of the fall varied between animals. In some instances the lowered intraocular pressure did not reach prestimulation levels and in other instances fell to levels far below initial pressures. Usually, the maximum fall occurred within 10 seconds after occlusion. The intraocular pressure then showed a very gradual rise and, if observed long enough, returned, in some cases, to the original elevated plateau.

In several experiments unilateral occlusion of the carotid artery induced a small rise of the elevated pressure in the contralateral eye and similarly a rise in blood pressure, most likely brought about by the carotid sinus reflex. Occlusion of the second carotid artery, in these experiments, not only reduced the intraocular pressure on that side, but also decreased the pressure on the previously affected eye to levels which were, as a rule, lower than those obtained upon unilateral occlusion. This sequence of events mainly illustrated the interplay of collateral circulation effects between the two carotid arteries shortly after ligation. Later, within 20 seconds, the intraocular pressure of both eyes rose gradually to varying levels. This pressure elevation was most likely brought about by influx of blood into the eyes from the

vertebral arteries. Release of the occluding ligatures resulted in restoration of the intraocular pressure to its original elevated values. Occlusion of the carotid arteries without electric stimulation affected the intraocular pressure in a similar way (fig. 8).

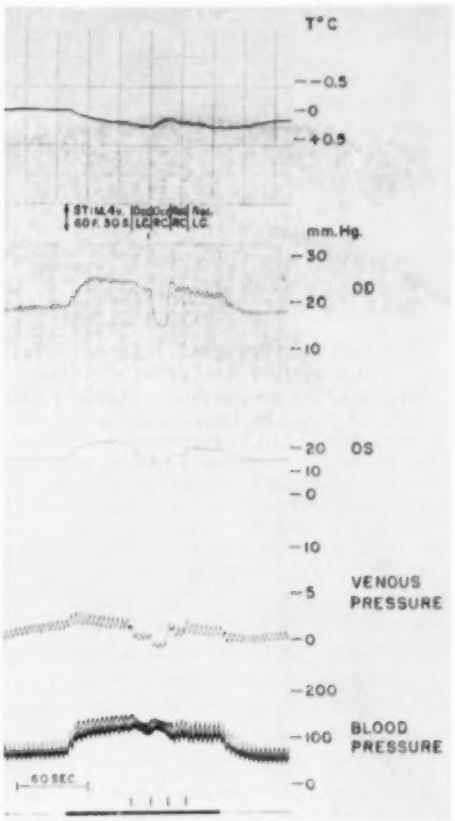


Fig. 7 (von Sallmann, et al.). *Influence of carotid artery occlusion during stimulation.*

Occlusion of the left carotid artery resulted in a fall of intraocular pressure (O.S.) Subsequent occlusion of the right carotid was followed by a steep fall of intraocular pressure (O.D.) and an additional fall of intraocular pressure (O.S.). Release of the carotid arteries restored the pressure plateau. The elevated temperature measured in the right eye was lowered by occlusion of the ipsilateral carotid artery. Pupils reacted fast and maximally and the nictitating membrane retracted slowly and incompletely, but neither could be observed during occlusion, because of manipulations in the neck. Position of positive electrode: 12 mm. anterior to and seven mm. above the interaural line. Pentothal anesthesia. (Occ) Occlusion. (Rel) Release. (LC) Left common carotid artery. (RC) Right common carotid artery.

Rises and falls of the intraocular pressure in these experiments were concomitant with elevation and lowering of the temperature in the subscleral space. During occlusion of the carotid artery, the blood column of some of the perilimbal vessels appeared narrowed. Although this series of experiments rendered information on collateral circulation between the carotid arteries on both sides and between this vascular bed and that of the vertebral arteries, the results were unrevealing as far as isolated responses of the intraocular vessels were concerned.

This aspect of the problem was studied in four experiments in which the blood flow through the carotid arteries was regulated, after the vertebral arteries had been ligated. As described in the technique, the abdominal aorta was connected to both common carotid arteries in a manner which allowed for maintenance of the head blood pressure at desired levels.

Electric stimulation in the ventral posterior hypothalamus produced elevation of the blood pressure and also of the intraocular pressure when the blood flow to the head was not interrupted. Gradual clamping of the catheter which joined the abdominal aorta and the common carotid arteries produced a fall of the intraocular pressure from the plateau produced by central stimulation (fig. 9). When the pressure in the tubing leading to the head was lowered to the prestimulation level, the continued central stimulus failed to maintain the intraocular pressure rise, although the aortic blood pressure responded in the usual manner.

Venous pressure recordings were obtained from the external and, in a few instances, from the internal jugular vein. In good preparations, they showed respiratory waves synchronous with those observed in the eye and blood pressure tracings. The venous pressure levels varied considerably between animals (1.0 to 8.0 mm. Hg). Centrally elicited changes of the venous blood pressure

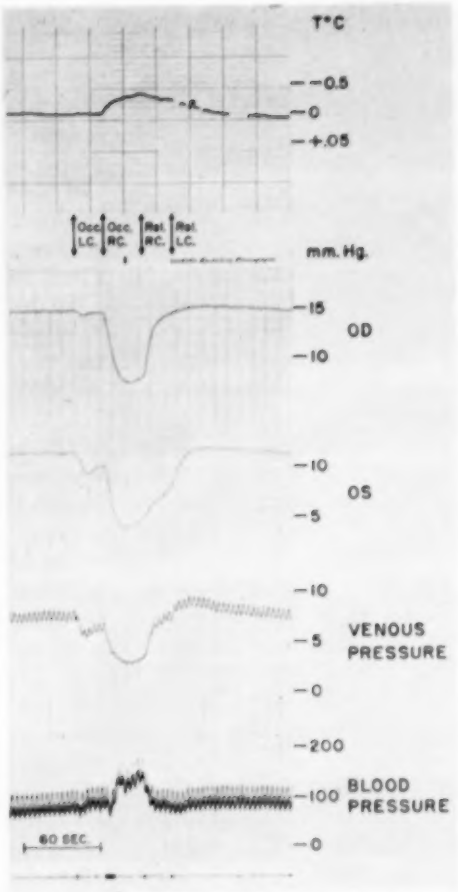


Fig. 8 (von Sallmann, et al.). *Influence of carotid artery occlusion without stimulation.*

Occlusion of the left carotid artery resulted in a fall of intraocular pressure (O.S.). Subsequent occlusion of the right carotid artery was followed by a steep fall of intraocular pressure (O.D.) and an additional fall of intraocular pressure (O.S.). The eye pressures then rose slightly before occlusion was terminated. Restitution of blood flow resulted in a steep rise of the pressure (O.D.) and a gradual rise of the pressure (O.S.). The same procedure on the left carotid artery re-established preocclusion intraocular pressure levels. The arterial blood pressure rise indicated the activation of the carotid sinus reflex. Fall of the venous pressure is probably due to the decreased arterial inflow to the head. Pentothal anesthesia. (Occ) Occlusion. (Rel) Release. (LC) Left common carotid artery. (RC) Right common carotid artery.

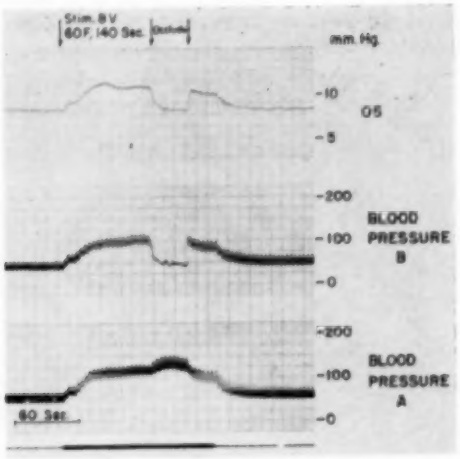


Fig. 9 (von Sallmann, et al.). *Influence of regulated blood flow to the head during stimulation.*

Lowering of the elevated carotid pressure (blood pressure, B) to base level by reducing the blood supply annulled the effect of stimulation on the intraocular pressure. Pupillary and nictitating membrane responses not recorded. Position of positive electrode: 12 mm. anterior to and seven mm. above the interaural line. Pentothal anesthesia. Blood pressure: (A) Aortic blood pressure. Blood pressure: (B) Effective carotid blood pressure.

were generally analogous to variations of the blood pressure and intraocular pressure, but the extent of the responses differed from animal to animal, and fell in the wide range of 0.5 to 9.0 mm. Hg. Moreover, the comparison of the venous blood pressure change and the increases in eye and arterial blood pressure did not disclose consistent quantitative relationships.

Effects of autonomic blocking and central nervous system depressant agents on hypothalamic-induced intraocular pressure rise

As a cholinergic blocking agent, atropine was used in a dose of 1.0 mg./kg. It did not change centrally evoked intraocular and blood pressure reactions (fig. 10). In a dose of 10 mg./kg. intravenous Priscoline (benzylimidazoline), an adrenergic blocking com-

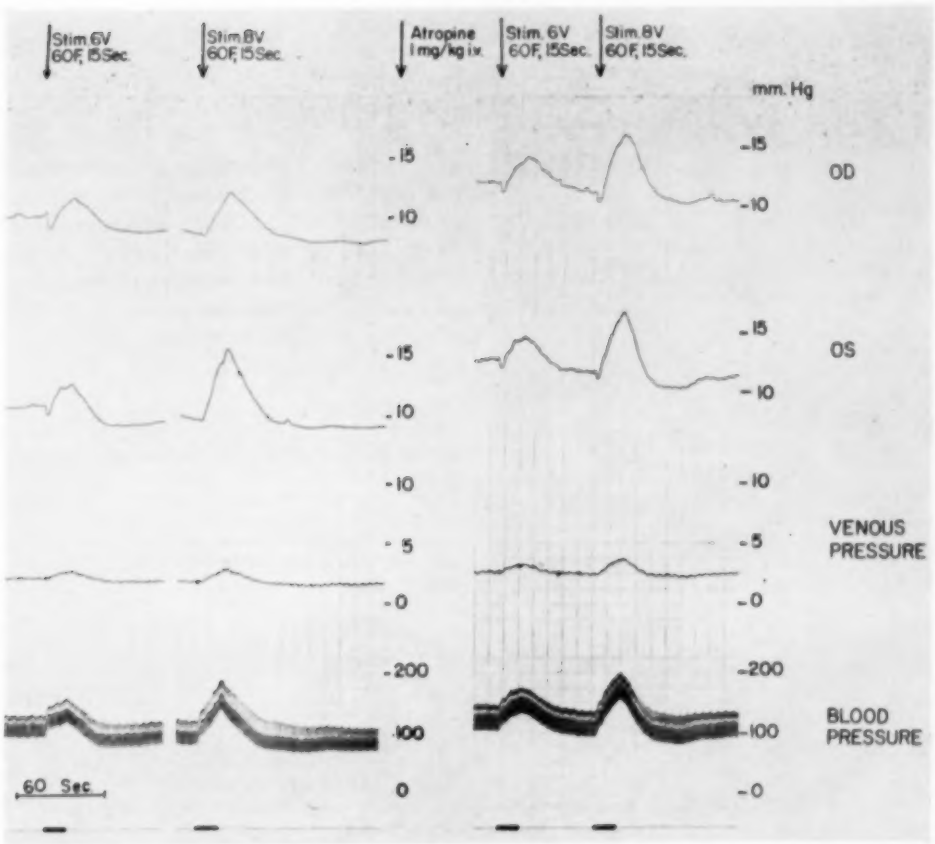
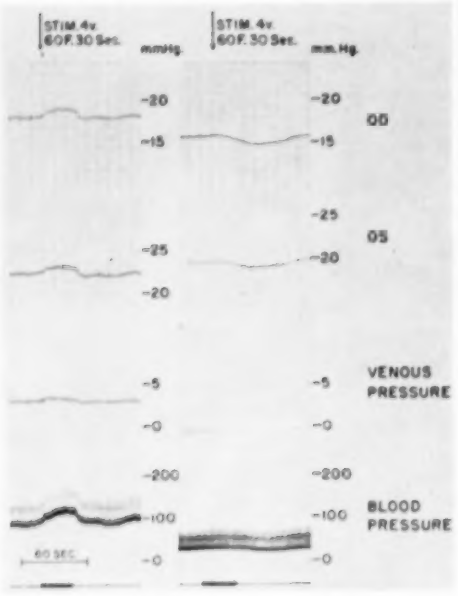


Fig. 10 (von Sallmann, et al.). *Influence of atropine.*
Atropine had no influence on the responses induced by central stimulation. One minute between plates A and B; 40 minutes between plates B and C. Position of positive electrode: 10 mm. in front of and six mm. above the interaural line. Chloralose anesthesia.

pound, almost completely abolished the induced eye and blood presure rises (fig. 11). Thorazine (chlorpromazine, dimethylamino-N-propyl chlorphenothiazine) (Fig. 12) and Sparine (promazine, dimethylamino-N-propyl phenothiazine) (fig. 13) also weakened or nearly extinguished such responses. Since chlorpromazine and promazine combine central depressant effects with adrenergic blocking action, the responsiveness of the preparation to noradrenaline was tested before and after the injection of the two pharmacologic compounds.

After injections of chlorpromazine (0.125 mg./kg. intravenous), characteristic elevations of eye and blood pressure, as seen after intravenous injections of one or 2.0 µg./kg. of noradrenaline, were greatly diminished, as were the effects of the central stimuli. Promazine reportedly acts as a central depressant similar to Chlorpromazine without some of its side effects. In the present experiments, promazine (0.5 mg./kg. intravenous) inhibited intraocular pressure re-



sponses to low-voltage stimuli. Such stimulation resulted in a reduced but not abolished pupillary dilation in contrast to the reactions of the nictitating membrane which were extinguished, even when stimuli of five volts were applied. An initial slight adrenergic blocking action was evident in the tracings of the blood pressure, but lasted only a few minutes. After this period, noradrenaline effects on blood pressure appeared somewhat potentiated, whereas the centrally evoked responses were greatly reduced at this time.

Fig. 11 (von Sallmann, et al.). *Influence of Priscoline.*

Priscoline completely abolished stimulation effects on blood and eye pressure. Initial mydriasis of the pupils forestalled observation of this response to stimulus. Nictitating membranes were removed. Position of positive electrode eight mm. anterior to and five mm. above the interaural line. Ether anesthesia.

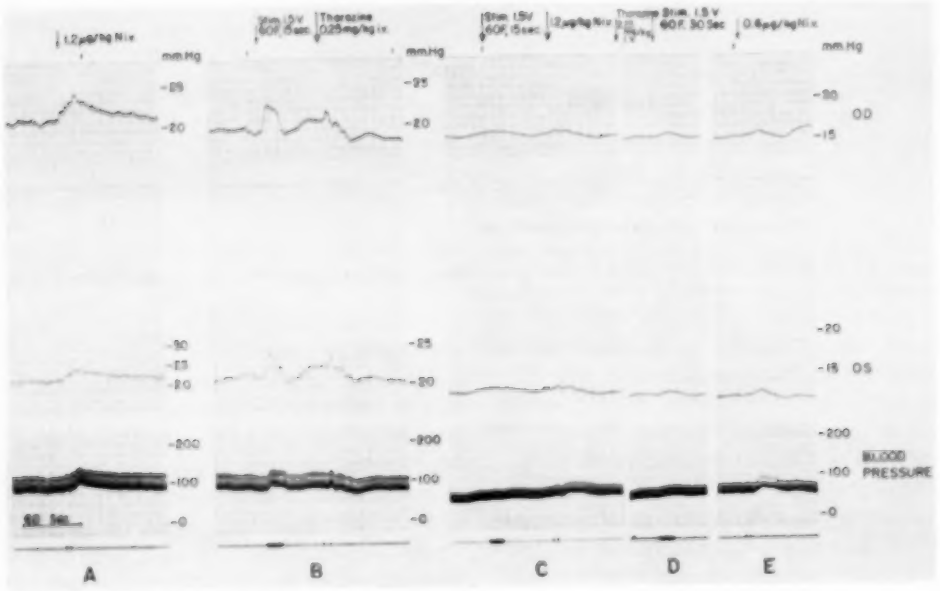


Fig. 12 (von Sallmann, et al.). *Influence of Thorazine.*

Thorazine inhibited the responses on the intraocular pressure and blood pressure induced by hypothalamic stimulation. After the injection, arterenol effects were initially depressed on both the intraocular pressure and blood pressure six min. between plates A and B; 22 min. between plates B and C; six min. between plates C and D; one min. between plates D and E. Position of positive electrode: 10 mm. in front of and six mm. above the interaural line. Chloralose anesthesia. (N) Arterenol.

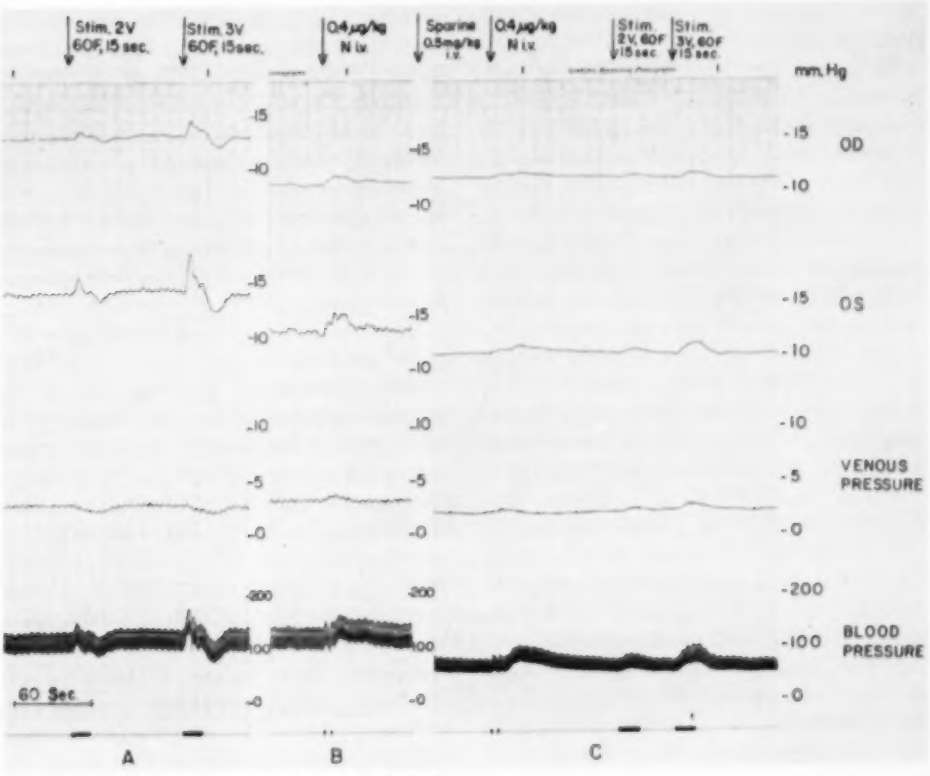


Fig. 13 (von Sallmann, et al.). *Influence of Sparine.*

Sparine inhibited the responses on the intraocular pressure and blood pressure induced by hypothalamic stimulation. After the injection, arterenol effects were initially depressed on both the intraocular pressure and blood pressure two min. between plates A and B; 10 min. between plates B and C. Position of positive electrode: 10 mm. in front of and seven mm. above the interaural line. Chloralose anesthesia. (N) Arterenol.

Stimulation of preganglionic fibers of the cervical sympathetic nerve produced retraction of the nictitating membrane, together with a rise of intracular pressure and pupillary dilation, in preparations which had received intravenous injections of promazine, indicating that sympathetic transmission of nerve impulses in the superior cervical ganglion was not blocked in these experiments.

Discussion

Previous investigations have shown that electric stimulation of certain areas of the diencephalon can give rise to complex response patterns of intraocular pressure, blood pressure, nictitating membranes, pupils, and peripheral blood vessels.[3] Depending on the site of stimulation, the studied effects were either closely correlated with each other in time and extent or relatively or completely dissociated. The present work focused on the intricate mechanism which governs typical eye pressure changes under the condition of the experiment. Selecting a stimulation point in the posterior ventral hypothalamus simplified the analysis of experimental results because of the predictable and reproducible nature of the effects. That is, in this area the difficult precise localization of the electrode tips were no prerequisite for elicit-

ing characteristic signs of general sympathetic excitation.

In the cat, the stimulation-induced acute intraocular pressure rise coincided with an increase of blood pressure, pupil dilation, and contraction of smooth orbital muscles. The latter are highly developed in this experimental animal as compared to man, and pressure changes resulting from their contraction are obviously of little interest when central nervous influences on eye pressure changes of primates are considered.

In a first approach, then, experiments were devised to separate neurovascular from neuromuscular effects. Sectioning of the cervical sympathetic nerve prior to or during central stimulation did in no way impede speed or diminish amplitudes of eye pressure rises, nor did they alter the grossly observed pupillary dilation. Unilateral excision of the superior cervical ganglion several days before the central stimulation procedure also did not interfere with the three reactions. On the other hand, no signs of smooth muscle contraction in the orbit were evident during the first 20 seconds of continued stimulation on the denervated side. Then the nictitating membrane retracted and lid fissures widened slightly, simultaneously with a small, slow increase of the mydriasis, thus the elicited smooth muscle contraction was not necessarily operative in the rapid eye pressure rise and apparently contributed little to the phenomenon under study.

The fact that stronger stimuli were required for a positive effect on the orbital smooth muscles than for eye pressure changes further confirmed this point. The delayed effects are explainable by an outpouring of adrenalin by the suprarenal glands as shown in the experiments on the pupil by Bender and Weinstein[6]; on the nictitating membrane by Magoun, Ranson and Hetherington[7]; and on blood pressure by Houssay and Molinelli.[8] The sudden eye pressure rise at the onset of stimulation concurred with a proportional increase of the blood pressure.

A series of experiments was concerned with the association of eye pressure elevation with blood pressure rises. Experimental evidence was sought to corroborate the impression that intraocular pressure rises were vascular in origin and were specifically caused by filling of the intraocular vessels due to increased blood flow. The measurements of the temperature at some distance from a densely vascularized area in the anterior segment of the eye were employed to examine this concept.

Thermometry had been used previously, with particular success, by Perkins,[9] who observed temperature rises up to 1.5°C. associated with an increased intraocular pressure upon stimulation of the fifth cranial nerve. In essence his technique of placing a fine thermocouple in the perichoroidal space was followed in the present work with slight modifications, especially in the recording of the effects. Simultaneous rises of temperature and eye pressure upon central stimulation indicated the importance of the vascular component in the reaction. Moreover, a synchronous fall of intraocular pressure and temperature ensued when the blood supply to the eye was intercepted.

The tracing of temperature changes showed retarded and more gradual responses when compared with eye pressure variations. Heat conduction and heat dissipation in the poorly vascularized tissue between thermocouple and the dense capillary network in the ciliary processes most likely brought about the delay of temperature responses in the subscleral space.

Biomicroscopic examination of the pericorneal vessels revealed that as a rule the vascular bed failed to react significantly to the described stimuli. This intimated a regional response of intraocular vessels to a central stimulus, but, at present, sufficient data are not available to distinguish between the responsiveness of various intraocular and extraocular vascular beds to stimuli elicited in the diencephalon.

Extraocular striate muscle activity, centrally induced, could not be ruled out by

electromyographic recording of action potentials because of stimulation artefacts. The inability of a myoneural junction blocking agent (decamethonium bromide) to inhibit these responses afforded sufficient evidence to exclude striate muscle contraction as the cause of eye pressure responses to stimuli in the ventral posterior hypothalamus. The observation of prolonged contracture of the extraocular muscle which followed the injection of decamethonium might have practical implications.

The problem of stimulation effects confined to intraocular vessels was the subject of another part of the study. In the acute experiments of this series, such an autonomous vascular response in the eye was not demonstrated. When the influence of systemic blood pressure changes was eliminated, electric stimulation in the diencephalon ceased to affect the intraocular pressure. These changes which occurred upon stimulation in the "sympathetic zone"[3] seemed to be subordinate to reactions of the general blood pressure, although the onset of eye response was somewhat delayed and the peak levels of the two pressures were reached at slightly different rates. For obvious reasons these observations are not applicable to the problem of the relative independence of eye and blood pressure base levels in general.

Another approach to the study of changes of the blood flow in the anterior segment of the eye consisted of counting Beta emissions from P^{32} labelled red cells which had been injected intravenously. Several properly curved probe counters were placed around the limbus in close proximity to the conjunctival surface. An Esterline-Angus milliameter unit connected with the scaler and rate meter served for recording of the ionization events. In preliminary experiments the counting plateau did not change significantly during the stimulation, despite the induced marked eye pressure increase.

Pressure rises in the jugular veins, which were observed during central stimulation, usually paralleled the changes of intraocular pressure and arterial blood pressure to various degrees. They indicated involvement of the venous system in the overall response of the peripheral circulation but with the presently employed technique did not clarify the influence of pressure changes in the ocular veins on the intraocular pressure. Planned continuous recording of the venous blood pressure in the anterior segment and in vortex veins might shed light on the influence of local blood flow on eye pressure variations.

The pharmacologic agents used in this study were cholinergic or adrenergic blockers and compounds of a complex action but predominantly with a central nervous system depressant effect. Whereas, atropine did not modify the responses under study, Priscoline prevented these reactions. The results, then, verified the sympathetic character of the stimulation effects. Chlorpromazine and promazine depressed or abolished the various responses, depending on the stimulation strength. Promazine and chlorpromazine apparently were capable of effecting this inhibition by a central mechanism, since blood pressure reactions to adrenergic mediators could be demonstrated during the period of drug activity.

A side issue of this study dealt with the differences between sensitivities of eye pressure, pupils and smooth muscles to excitation evoked in the posterior ventral hypothalamus. Intraocular pressure and pupils reacted to similar threshold stimuli, in contrast to the smooth orbital muscles, which required higher voltage. These results are tentative, however, because tissue injury produced by electric current of higher voltage may interfere with the reproducibility of response and obviate proper interpretation of data obtained in experiments in which stimulations had been effected repeatedly. Further work with a biphasic type of stimulus will provide better experimental conditions to quantitate systematically the different sensitivities of the effector systems in the eye or orbit.

The surprising observation of an un-

changed reaction of the pupils when the sympathetic nerve has been sectioned prior to or during stimulation also needs re-examination with an improved technique, that is, with pupillography. To the naked eye, pupil responses after severing of the nerve did not appear slower and therefore did not indicate inhibition of the third nerve nucleus as the only underlying mechanism of pupil dilation.* In this context mention should be made of the fact that sectioning of the vagi did not visibly change the pupillary or other responses of the eye to electric stimulation in the hypothalamus. This observation rules out the possibility that sympathetic impulses, effecting these reactions, travelled in fibers which anatomically connect the vagus and cervical sympathetic nerves.[10]

In the present series, the intraocular pressure responded to the described central stimuli regularly with a rise of the intraocular pressure. Stimulation of the cervical sympathetic nerve, on the other hand, resulted frequently in a fall of this function similar to that produced by small doses of adrenalin. In other preparations the stimulation of this nerve, however, was followed by a rise of the intraocular pressure. This discrepancy in stimulation effects remains unsolved. Varying frequency or voltage of the stimulus applied to the sympathetic nerve did not seem to change the type of response, nor was it possible to show that the form of response depended on the pre-existing eye pressure level.

Summary

1. The reproducibility of associated responses which ensued from electric stimulation in the posterior ventral hypothalamus of the cat rendered these reactions suitable for systematic analysis of underlying mechanisms. The positive reactions in the present study comprise rises of the arterial, venous, and intraocular pressure; pupillary dilation; contraction of the smooth orbital muscles; and, occasionally, fleeting changes in the pericorneal vascular bed.

2. Severing of the cervical sympathetic trunks and the vagi or antecedent excision of the superior cervical ganglion did not impede the stimulation effects of eye and blood pressure or of the pupils, did abolish contraction of smooth orbital muscles temporarily. Thus, smooth muscles did not contribute noticeably to the acute centrally produced eye pressure rises.

3. By means of a muscle relaxant (decamethonium) it was possible to rule out the participation of extraocular striate muscles in the described responses of the intraocular pressure.

4. Elevation of temperature in the anterior subscleral space during stimulation accompanied the intraocular pressure rises, and falls of temperature followed occlusion of the the carotid vessels. Thus, therometry served as an aid in determining changes of intraocular blood flow and indicated that centrally evoked increments of ocular pressure resulted from increased blood flow.

5. The rapid rise of intraocular pressure which occurred upon stimulation in the ventral hypothalamus was shown to be subordinate to the co-existing elevation of systemic blood pressure; the intraocular pressure failed to react to central stimuli when the influence of increased blood pressure was annulled.

6. Delayed additional rise of intraocular pressure, augmentation of the mydriasis, and moderate retraction of the denervated nictitating membrane, when central stimulation was continued for more than 20 seconds, was apparently mediated by the outpouring of adrenalin from the suprarenal gland.

7. Autonomic adrenergic blocking agents and central depressants reduced or extinguished the signs of central sympathetic stimulation on the eye. The probable mechanisms of these phenomena were discussed.

National Institutes of Health (11).

* Pupillary dilatation resulting from hypothalamic stimulation was studied pupillographically by I. R. Loewenfeld.[9]

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Discussion

Dr. W. Morton Grant (Boston): This is indeed a most elegant arbeit. The most modern means have been employed to obtain information simultaneously on a number of factors involved in a very complex reaction. The techniques and interpretation of results can scarcely be criticized. The evidence seems convincing to me that a rapid increase of intraocular pressure by a few mm. mercury is caused by electrical stimulation of the hypothalamus in cats, that this response is mediated by sympathetic nerves which are as yet unidentified, and that the response is largely an intraocular vascular one subordinate to elevation of the systemic blood pressure.

Although the authors have already pointed out that their study was concerned only with transitory effects of short periods of stimulation and that they were not concerned with central nervous influences on aqueous dynamics, it is hard to refrain from considering the possible significance of these findings in relation to the hydrodynamics of the eye. Indeed, if the change in intraocular pressure which they observe is a reflection only of a change in intraocular blood-volume, no sustained influence on intraocular pressure is to be expected. However, if further study with prolonged stimulation should show that the vascular response caused a change in facility of outflow or rate of formation of aqueous humor, then the possibility of real central nervous control over steady-state intraocular pressure would be demonstrated. Speculating on the implications with respect to glaucoma, it seems evident that the phenomenon studies by Dr. von Sallmann and his associates could have little significant influence in glaucoma of the open-angle variety unless a mechanism for sustained alteration of intraocular pressure were found. On the other hand, in glaucoma which is caused by the closing of an abnormally narrow angle, it is quite intriguing to speculate on the possibility that this intraocular vascular phenomenon associated with pupillary dilation could constitute the mechanism for precipitating angle closure in response to strong emotional stimuli.

Dr. Ludwig von Sallmann (closing): I would like to thank Dr. Grant for his learned discussion. It was the comment of an expert on problems dealing with the physiology of the intraocular pressure.

We shied away, as you know, from drawing any conclusion with respect to eye pressure pathology because it was felt that the experiments were carried out under conditions quite different from those which might operate in the human eye of unanesthetized patients. We do plan to start prolonged stimulations at low voltage with bi-directional wave forms and hope that the influence of such stimuli on aqueous dynamics may provide some leads.

Analysis of dissociated responses was not included in the present study because, as a basis for further work, it was desirable to have reactions which can be produced with great regularity. The stimulation points from which isolated changes of the intraocular pressure can be elicited in the dorsal hypothalamus can not be defined by the coordinates of the Horsley-Clarke stereotaxic instrument. It would be necessary, therefore, to sacrifice a great many animals without obtaining results from such experiments. Present experiments are extended to parts of the midbrain. Here dissociated responses appear to be provoked more frequently; we plan to select such stimulation points for studying isolated responses of the intraocular pressure.

A STUDY OF THE INNERVATION OF THE CHAMBER ANGLE*

MONTE G. HOLLAND, M.D., LUDWIG VON SALLMANN, M.D.,
AND ELEANOR M. COLLINS
Bethesda, Maryland

The search for nervous pathways and nerve endings as mediators of impulses for the regulation of the intraocular pressure is limited in this study to functionally important structures in the chamber angle, namely to the trabeculum, Schlemm's canal, and collector channels. The purely morphologic approach followed in this work is timely since it provides detailed anatomic data on nervous elements in an area, the physiology and pathology of which has been brought into focus in recent years by many investigators.

MATERIAL AND METHODS

The material studied comprised four groups of vertebrate eyes, namely, of man, monkey (Rhesus), chicken (Rhode Island Red), and rabbit (albino). Seven human eyes were studied by the Gros-Schultze and Bodian silver impregnation techniques. The Gros-Schultze method was employed on frozen sections in planes parallel and perpendicular to the limbus. Paraffin embedded material sectioned in the same planes served for the Bodian-Protargol procedure. The sections which contained the trabeculum and Schlemm's canal were stained and examined in series. The same techniques were used on 17 monkey eyes. In addition, the Glees silver method[1] and Peter's[2] technique were given a trial in this species. In processing chicken eyes (three eyes), a decalcification in formic acid preceded the embedding in paraffin since the scleral wall in this species contains ossicles in the region of the limbus. Serial sections were obtained again in tangential and sagittal planes.

Eighteen eyes from albino rabbits were subjected to vital staining with methylene blue, according to the method described by Weddell, et al.[3,4] which was modified as follows:

Hyaluronidase (Aldase of Searle Company) was dissolved in a 0.9-percent solution of sodium chloride; 0.05 to 0.1 cc. of this solution, containing 5.0 to 10 TR units, was injected into the anterior and posterior chambers of the anesthetized animals. The eyes were gently massaged for 20 minutes. Then followed the injection of 0.1 cc. of a 0.04-percent solution of methylene blue (prepared in 0.9-percent solution of sodium chloride) into the anterior and posterior chambers. Leakage and replacement of the injected dye by aqueous humor occasionally necessitated one or two reinjections of the methylene blue solution. The time allowed for intravital staining was usually 30 minutes and occasionally 45 minutes.

At the end of this time the animals were killed with an overdose of sodium pentobarbital, the eyes enucleated, and bisected in a medial plane. The two halves were then flattened and exposed to air for approximately 30 minutes. The intensity of staining of nerve fibers was observed under the ×40 magnification of a Zeiss otoscope. For visualization of the trabecular zone and in order to minimize the trauma, the iris was gently reflected back from the angle under direct observation through a dissecting microscope.

When the optimal degree of staining had been reached, the preparations were flattened between two glass slides, placed in eight-percent Ammonium molybdate solution, and kept under refrigeration overnight. They were then washed in cold running water for one to two hours; dehydrated in three or more changes of tertiary butyl alcohol for at least 24 hours; cleared in several changes

* From the Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Maryland.

of xylol; and mounted in Permount as whole preparations.

Other techniques for intravital methylene-blue staining were also tested, such as intracarotid perfusion and perfusion combined with local injection. Four rabbit eyes which had been stained vitally with methylene blue were sectioned from paraffin embedded and frozen material. In some experiments other fixatives and dehydrating procedures were tried but the best results were obtained with the technique outlined above. The silver techniques of Gros-Schultze and Bodian were used on four rabbits and results were compared with the methylene blue stained preparations. Finally to confirm the specificity of the silver impregnation procedures for nerve fibers, the von Pap reticulum stain was applied as a control to sections of human, monkey, and chicken eyes of the same series.

Results

The observations are grouped according to the species examined. Silver-stained material served for the examination of human, monkey, and chicken eyes. The innervation of the rabbit chamber angle was studied on methylene blue and silver stained preparations. Examination of whole mounts provided the most convincing information.

Origin, course, and termination of nerves in the trabecula of the human eye

The nerves in the region of the trabecula and Schlemm's canal appear to arise from the supraciliary and ciliary plexus in the region of the scleral spur (fig. 1). Tangential sections through this area show that, as a rule, the stem axons lie in the region of the scleral spur, either parallel to the limbus or, occasionally, perpendicular to it. From these stem axons smaller branches derive, which course into the trabecula. They pursue a somewhat sinuous path and divide into still smaller branches, often at right angles to the parent fiber. Sometimes the fibers form loops, reversing their direction, but the general pattern is that of terminal arborization in which subsequent axonal divisions give rise to progressively smaller fibers (fig. 2).

Figs. 1 and 2 (Holland, von Sallmann, and Collins).

(1) Plexiform arrangement of nerves in trabecula, showing origin of trabecular nerves from the ciliary plexus. (Human, Bodian silver stain, ×285.) Trabecula (tr.); scleral spur (s.s.); ciliary body (c.b.).

(2) Terminal arborization in trabeculum. (Human, Bodian silver stain, ×1,760.)

The fine endings, demonstrable only with high magnification, seem to represent free axonal filaments and do not show constant anatomic relationship to the endothelial cells covering the trabeculae, but terminate freely on the trabecular lamellae (fig. 3).

Occasionally, terminal enlargements of the axonal filaments are noted. Sometimes fibrillar or basketlike varicosities are visible along the course of axons, and rarely at their terminations (fig. 4). All fibers which end in the trabecula are simple, free axonal filaments and not specialized endings. The general pattern of the innervation of this region, then, is that of a plexiform arrangement of delicate arborizing axons which are interwoven in various planes with other fibers but do not appear to fuse.

In the human eye all parts of the trabecula contain nerves and terminal filaments, but the region adjacent to the scleral spur gives the impression of being slightly richer in nerves than the apex of the trabecula at its insertion on Descemet's membrane. Schwann cell nuclei are observed frequently along preterminal fibers and on some of the finer terminal axons.

Innervation of Schlemm's canal and collector channels in the human eye

Since the trabecular meshwork forms the inner lining of Schlemm's canal, it is not surprising that nerves and free nerve endings occur in juxtaposition to the endothelial lining of the canal. Not infrequently, slender nerve fibers can be seen to approach the wall of Schlemm's canal and to terminate there as very fine axonal filaments (fig. 5). The topographic relationship of the terminal axon to the wall of the canal varies, as it may end perpendicular or parallel to the lumen. Sometimes the terminal fiber approaching from the trabecular side is separated from the lumen of Schlem's canal by two or three layers of endothelial cells which cover adjacent trabeculae. At other times, axons appear to penetrate to the endothelium of the canal and terminate near the base of these cells (figs. 6 and 7).

Frequently the terminal filaments around Schlemm's canal arise from nerves located in the trabecula or in the area of the scleral spur, but they may also originate from nerves situated in the adjoining scleral tissue. Occasionally, preterminal axons are seen to divide just beneath the endothelium of Schlemm's canal at the outer scleral wall (fig. 8), and to follow a looped course adjacent to the wall, curving over its apex to the trabecular side and ramifying irregularly into fine branches. In short, then, many preterminal nerve fibers have close topographic relationship to both the inner and outer wall of Schlemm's canal. Some of these give off delicate axonal filaments which end beneath the endothelial lining as free nerve endings.

The nerves of the collector channels which connect Schlemm's canal and the scleral venous plexus consist of fine axonal terminals; they end in the connective tissue around the channels a short distance from

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Figs. 3-8 (Holland, von Sallmann, and Collins).

(3) Free nerve ending in trabecula. (Human, Bodian silver stain, ×1,660.)

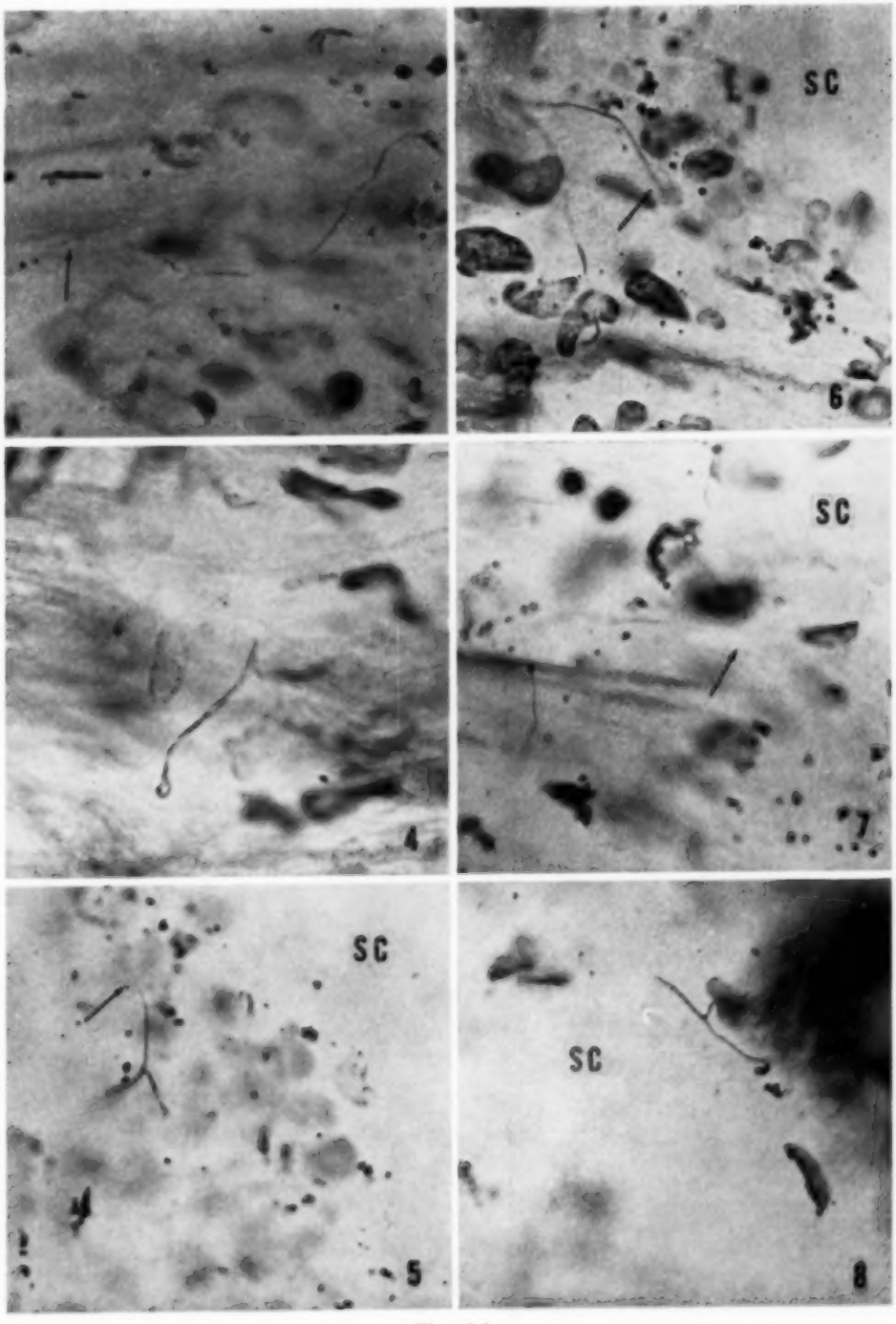
(4) Axonal vesiculation of terminal in roof of Schlemm's canal. (Human, Bodian silver stain, ×1,660.)

(5) Free nerve ending in relation to inner wall of Schlemm's canal (S.C.). (Human, Bodian silver stain, ×1,660.)

(6) Preterminal axon giving off subendothelial terminal in relation to Schlemm's canal (S.C.). (Human, Bodian silver stain, ×1,380.)

(7) Free nerve ending just beneath the endothelial lining of Schlemm's canal (S.C.). (Human, Bodian silver stain, ×1,660.)

(8) Axonal division beneath the endothelium of Schlemm's canal (S.C.). (Human, Bodian silver stain, ×1,660.)



Figs. 3-8

the lumen.* Terminal arborizations in the walls of the collector channels themselves were not observed in the preparations studied.

Innervation of angle of monkey eye

The innervation of the trabecula, Schlemm's canal, and collector channels in the monkey eye is similar to that in human eyes (figs. 9, 10, 11, and 12). In this species, however, the base of the trabecula near the scleral spur appears to be much richer in nerves than its apex. Another anatomic feature which seems to be developed more prominently in the monkey is represented by connective tissue protrusions or cushions bulging into the lumen of the canal; occasionally they contain preterminal axons (fig. 13).

Comments on the innervation of the angle of the chicken eye

In view of the relatively small number of chicken eyes studied (although about 500 serial sections were examined), the description of the innervation of this region is tentative and contingent on more extensive studies in the future. The region of the corneoscleral junction, visualized in the plane parallel to the layer of loosely disposed connective tissue in which the sinus venosus sclerae is located, is very rich in nerves. Many of these are thick fibers, bound together into bundles, which traverse the area to reach Crampton's muscle. The walls of the sinus are encircled by coarse and fine nerve fibers. Free nerve endings are present in the adventitial wall of the sinus (fig. 14). The largest of its divisions lies close to the inner surface of Crampton's muscle. A great number of the coarse fibers in this region lead to muscle bundles and neighboring structures. In the sections examined, the pectinate fibers appear to be devoid of nerves with the exception of an occasional nerve loop or preterminal axon at the base of some of the pectinate fibers.

Innervation of the trabecula of the rabbit eye

The general distribution of the nerves in the trabecular area, as demonstrated in silver-stained material of the rabbit eye, shows striking similarity to that of the primate eye. Preterminal axons arise from branches of the ciliary plexus, enter the region of the trabecula, and end among endothelial cells as free axonal filaments (fig. 15).

Observations made on eyes which were subjected to intravital methylene-blue staining, resemble, in most respects, those obtained on silver-stained sections (fig. 16). New information on neurohistologic details emerged from the study of whole mounts of rabbit eyes which had been stained intravitally with methylene blue.

Corneal, trabecular, and iridociliary nerve fibers appear to have a common origin (fig. 17). As the large ciliary nerves approach the cornea from the sclera and the supraciliary space, they divide just proximal to the iridocorneal angle and give off branches to the ciliary body and iris. Before they reach the margin of the cornea they frequently

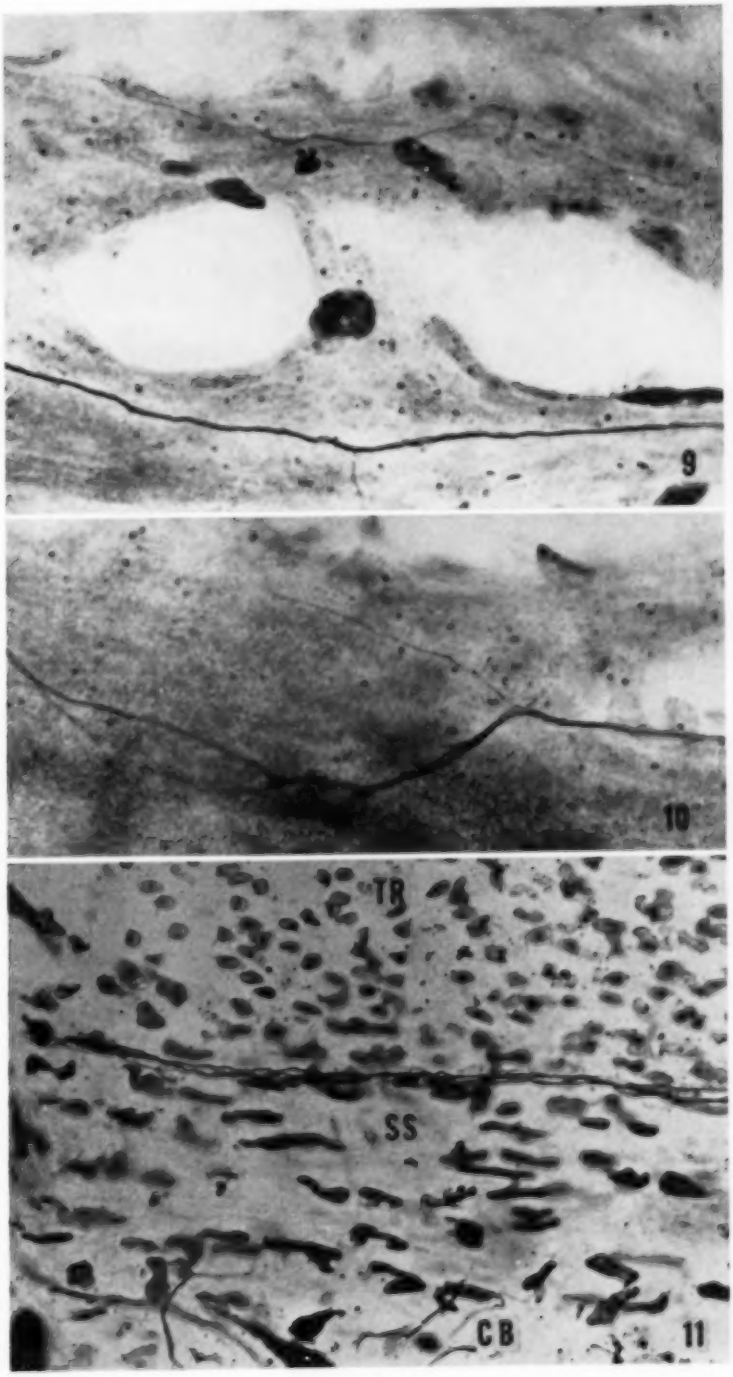
* The innervation of these channels is similar to the innervation of capillaries described by Weddell and Pallie.[8]

Figs. 9-11 (Holland, von Sallmann, and Collins).

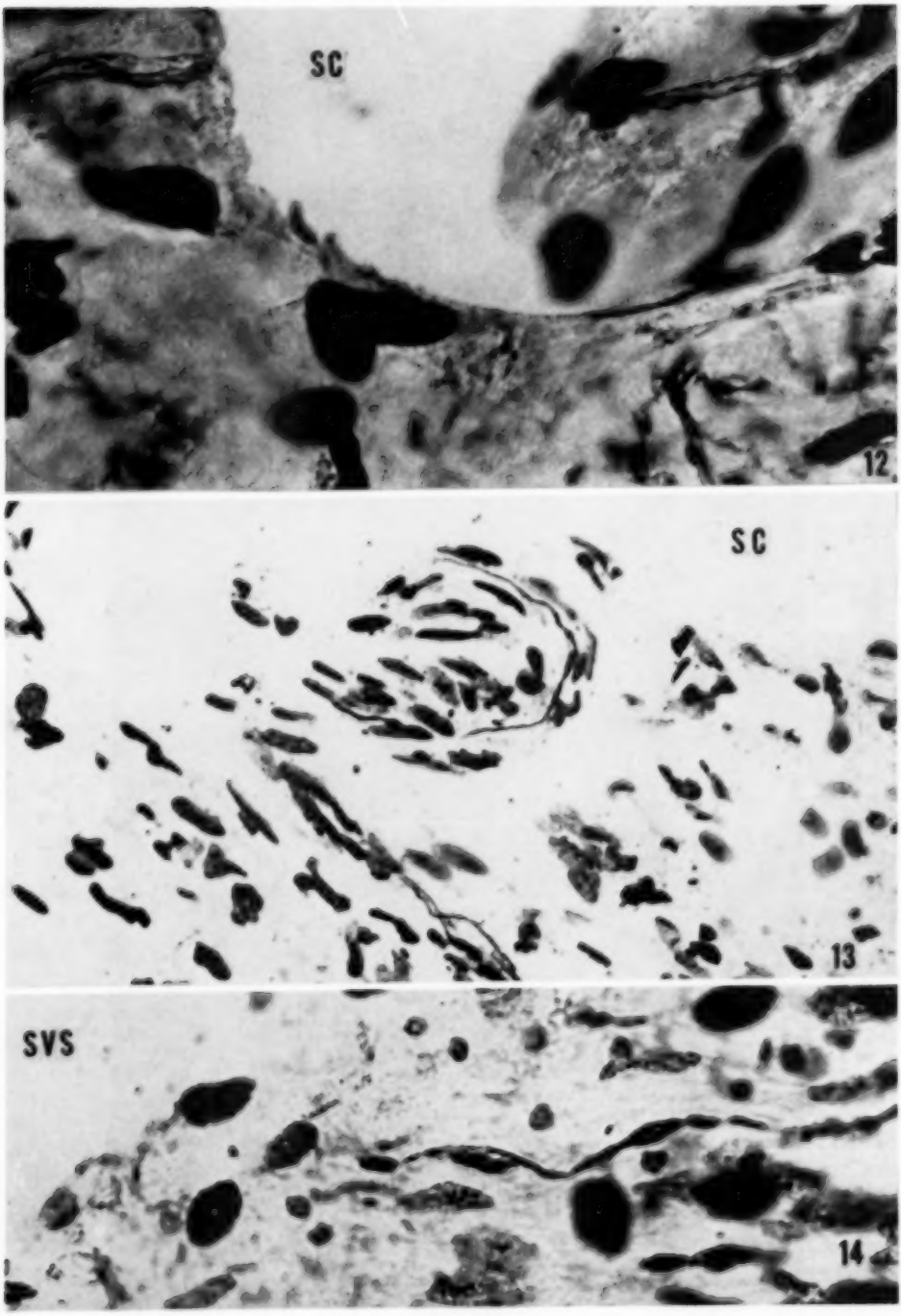
(9) Below: preterminal axon giving off fine branch at right angles. Above: very fine free axonal filament ending on wall of trabecular lamella. (Monkey, Gros-Schultze, ×1,760.)

(10) Terminal arborization in trabecula. Free nerve ending among endothelial cells. Many of the dark spots are nucleoli. (Monkey, Gros-Schultze, ×1,760.)

(11) Large bundle of preterminal axons at base of trabecula. In other sections this bundle was seen to arise from the ciliary plexus. (Monkey, Bodian silver strain, ×380.) Trabecula (tr.); scleral spur (s.s.); ciliary body (c.b.).



Figs. 9-11



Figs. 12-14

branch into rami which run close to the outer surface of the trabeculum (fig. 18) and arborize into a rich plexus of delicate fibers. Some of the nerve trunks which enter the corneal stroma proceed in a course parallel to the limbus and send off branches to the trabeculum, forming terminal arborizations. Other fine branches and terminal fibers in the trabecular meshwork originate from nerves which appear to supply mainly the iris and ciliary body.

Thus two systems of fibers participate in the innervation of the trabecula. One group is derived from the fibers which supply the cornea and the other group stems from the iridociliary plexus (figs. 19 and 20). Analogous to observations on the cornea (Weddell and Zander[3]), the terminal arborizations in the trabeculum consist solely of free nerve endings. This type of termination is also seen in the ciliary body and iris. None of the eyes examined showed specialized encapsulated or unencapsulated neural "end-organs." Under high magnification, the vitally stained nerve branches taper off into very fine beaded naked axons (fig. 21). These terminal filaments do not show consistent relationship to endothelial cells.

Discussion

The ultimate structure of nerve terminals in the autonomic nervous system is still a controversial issue. Most authors today agree, however, that the terminal fibers are arranged in a plexiform pattern[6, 7] and, as a rule, end freely. Specialized endings have been described in organs supplied predominantly by autonomic fibers, but the origin and function of these fibers are obscure.[8, 9] According to Fulton[8*] "autonomic visceral afferents do not imply any functional peculiarity of the afferents as compared with somatic afferents. Actually the sensory innervation of the visceral cavities is derived from both autonomic and somatic afferent systems." Observations in the present study suggest that the innervation of the trabecula, like that of visceral cavities, may arise from these two systems of fibers.

In the peripheral somatic afferent system the nerves terminate as free endings or as encapsulated specialized endings. Recent work has indicated that in the hairy skin there are only free endings,[10] while in the skin of the glabrous areas, various forms of specialized endings can be seen.[11] Weddell and Zander,[3] in their study of the innervation of the cornea, have shown convincingly that in this tissue only free endings are present.

Previous studies concerned with the innervation of the angle of the anterior chamber have not provided convincing information regarding the presence of endings in this region and their histologic type. Boucheron,[12] who examined this region in the eye of the guinea pig by the gold-chloride technique, described nerves spirally disposed around the pectinate fibers and axons in a "Gothic arch" arrangement, but no mention was made of the presence of terminals.

Kolmer[13] demonstrated with the silver method of Agduhr numerous nerves in the region of the angle and Schlemm's canal, but was not able to show endings in this area and thought that the nerves were fibers en passage.

Most recently Vrabec[14] examined the angle of four different vertebrate eyes by means of the Gros-Schultze silver technique. He described the innervation of the human trabeculum as arranged in a lattice pattern

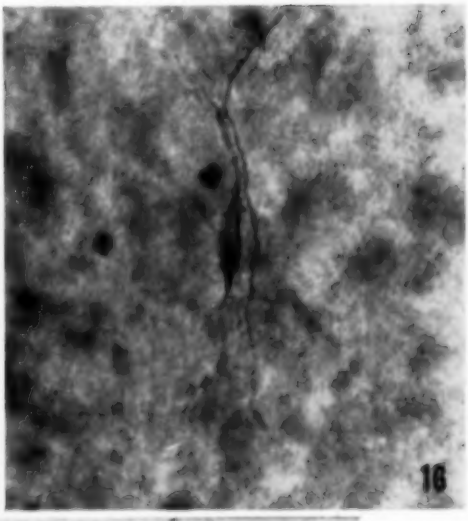
* Fulton, page 387.

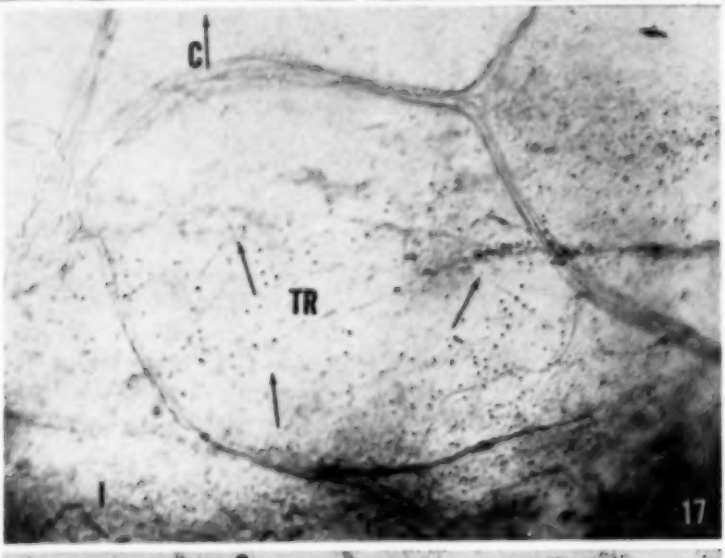
Figs. 12-14 (Holland, von Sallmann, and Collins).

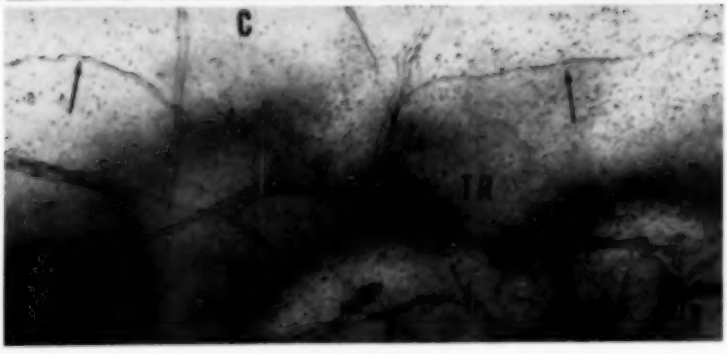
(12) Free nerve endings in wall of Schlemm's canal (S.C.). (Monkey, Bodian silver stain, ×1,750.)

(13) Connective tissue protrusion in Schlemm's canal (S.C.), containing preterminal axon. Axons in relation to wall of Schlemm's canal. (Monkey, Bodian silver stain, ×600.)

(14) Subendothelial nerve ending in wall of sinus venosus sclerae (S.V.S.). (Chicken, Bodian silver stain, ×1,250.)







Figs. 15-18

and nerve endings as loops or free terminals. The published illustrations convey the impression that the nerve branches are elements which pass through the trabecular region. In one goose eye, Vrabec noted and photographed a Herbst corpuscle in the trabeculum. He also mentioned briefly the presence of nerve fibers penetrating the wall of Schlemm's canal but did not give further details.

In the present study several silver-impregnating techniques and the intravital methylene-blue staining method were employed. Frozen and paraffin embedded material was sectioned serially to follow the course of neural elements and whole mounts of bisected eyes were examined microscopically. None of these methods gave the slightest indication of the presence of organized peripheral nerve endings in the examined structures of the anterior chamber angle of primates, rabbits and chicken eyes.

The significance of these results becomes clear when the structure of the nerve terminals is correlated with the function or "modality" which they subserve.

The theory that various primary "modalities" of sensation are associated with morphologically specific nerve endings stems from the work of von Frey,[15] and bears the name of "punctate sensibility." The theory states that for each primary sensation there is a morphologically identifiable specific nerve terminal or "end organ" which subserves this sensation. For example, free nerve endings are said to be specific for pain; sensations of warmth or cold are mediated by corpuscles of Ruffini and Krause, and that of pressure by Pacinian corpuscles, and so forth. The recent work of Sinclair, Weddell, and Zander[16] and Hagen, Knoche, Sinclair, and Weddell[17] has shown that there is no correlation between the presence of the various primary modalities of cutaneous sensation and the presence of morphologically specific nerve endings.

In over 200 biopsies of human skin in which cutaneous sensibility had been tested prior to biopsy, Dastur[19] observed only free nerve endings in both the perifollicular nerve baskets and in the intrafollicular nerve nets. Lele and Weddell[18] have shown recently that in the cornea where there are only free nerve endings present, thermal, touch, and painful sensations may be elicited from its central portion. Thus it appears that morphologically indistinguishable free nerve endings may mediate a variety of sensations.

This concept is pertinent to the problem of demonstrating receptor terminals within the angle of the anterior chamber which could act as mediators in the regulation of the intraocular pressure. Since in the light of current knowledge unorganized free endings serve a variety of sensory "modalities," specialized receptors mediating impulses for eye pressure regulation appear unnecessary. As an analogy, the innervation of pressure receptor areas in various hollow viscera, such as in the aortic arch, carotid bodies, great veins, and so forth, is represented by a plexiform arrangement of highly branched nerve fibers with undifferentiated free endings.[6, 8]

No conclusions can be drawn on purely histologic grounds as to the function of the nerves and nerve endings described in the trabecula. The fact that some of the terminal fibers in the trabecula branch off from cor-

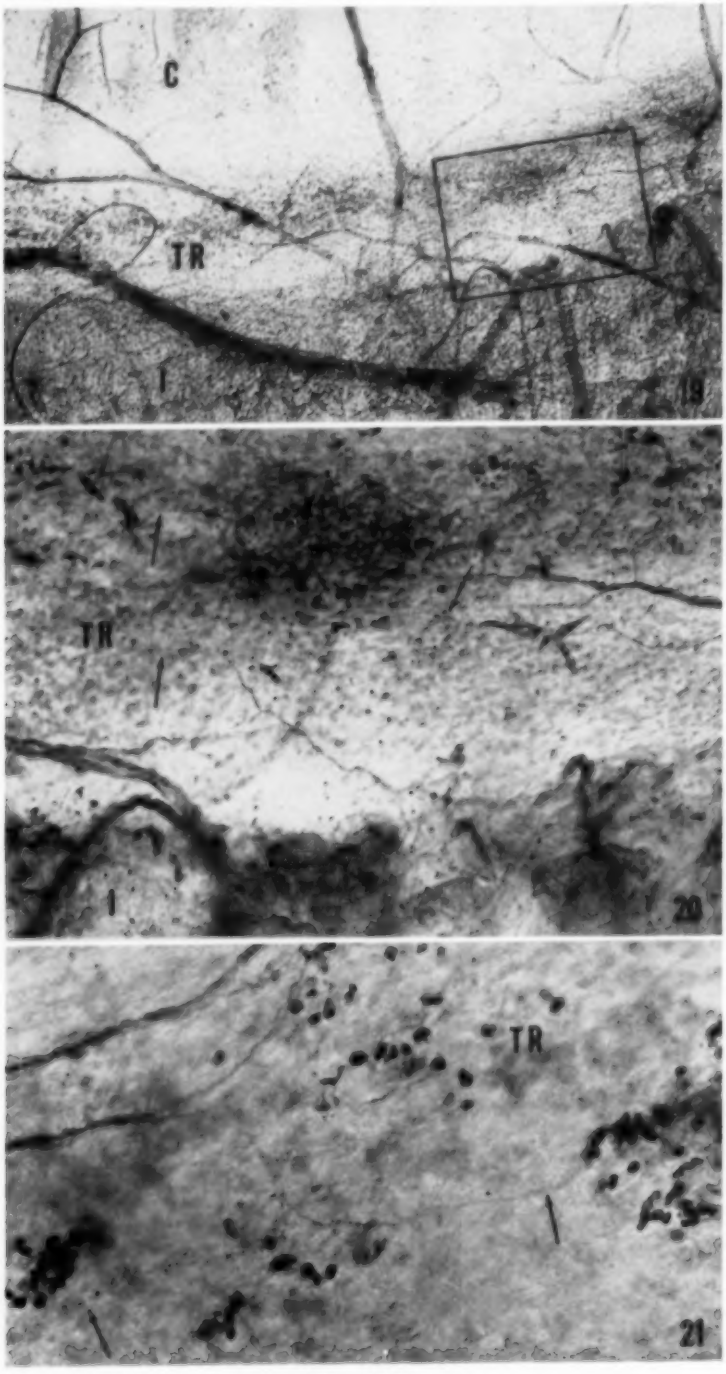
Figs. 15-18 (Holland, von Sallmann, and Collins).

(15) Free nerve endings in the trabecula of the rabbit eye originating from ciliary plexus. (Bodian silver stain, ×1,660).

(16) Appearance of free ending in rabbit trabecula stained vitally with methylene blue. (×515.)

(17) Demonstrates common origin of corneal, iris, and trabecular nerves. (Rabbit, methylene blue vital stain, ×105.) Cornea (c); trabecula (tr.); iris (i). Arrows show delicate endings in the trabecula.

(18) Trabecular rami arising from nerve trunk just before it enters the cornea. (Rabbit, methylene blue vital stain, ×73.) Cornea (c); trabecula (tr.); iris (i).



Figs. 19-21

Figs. 19-21 (Holland, von Sallmann, and Collins).

(19) Low-power view of innervation of rabbit angle. Components from two systems of fibers are visible. (Rabbit, vital methylene blue, ×30.) Cornea (c); trabecula (tr.); iris (i).

(20) Higher-power view of blocked area in Figure 19, showing free nerve endings in the trabecula from branches supplying the cornea (upper two arrows) and from branches which innervate the iris (arrow). (Methylene blue vital stain, ×105.) Trabecula (tr.); iris (i).

(21) Free endings in trabecula. Fine beading of the delicate terminal axons is visible. (Rabbit, methylene blue vital stain, ×285.) Trabecula (tr.).

neal nerves, a priori, suggests their classification as somatic afferents. Of particular interest in this respect are those fine fibers which originate from nerves within the corneal stroma and take a retrograde course to the trabecula, where they end. However, the ciliary nerves, from which the corneal branches arise, contain both autonomic afferent and efferent fibers, as well as somatic afferents. Thus the described anatomic relationship alone does not provide a basis for a clear differentiation of these fibers.

The reported observations leave little doubt on the participation of autonomic fibers in the innervation of the trabecular region. It is not clear whether or not efferent fibers also participate in the nerve supply to this area. Planned degeneration studies may contribute to the distinction between parasympathetic and sympathetic components and to the question of whether truly somatic afferent fibers are present in the plexiform arrangement of axons in the area of the trabeculum, as suggested by this study.

Summary and Conclusions

1. The innervation in the region of the chamber angle was studied in four classes of vertebrate eyes. The use of several silver-impregnation techniques, with serial sections on frozen and paraffin embedded material, together with whole mounts of the anterior segment stained vitally with methylene blue, permitted a detailed examination of the origin, course, and termination of nerve fibers in this region.

2. The innervation was shown to consist of a plexiform arrangement of delicate preterminal and terminal axons which were interwoven in different planes and ended as free axonal filaments.

3. Axons terminating in this region originated from two systems of nerve fibers, the iridociliary plexus and the nerves which supply the cornea.

4. Free nerve endings were shown beneath the endothelium of Schlemm's canal and within the trabecular meshwork that forms the inner wall of Schlemm's canal.

5. In the human, monkey, rabbit, and chicken eyes, no encapsulated or specialized nerve endings were observed in the area of the angle. In the chicken eye free nerve terminals were demonstrated in the adventitial wall of the sinus venosus sclerae.

6. The histologic observations are discussed on the basis of the current concept of the relation of nerve endings to their function and attention is directed to the possible role of nerve fibers and endings in the regulation of intraocular pressure.

National Institutes of Health (11).

We are greatly indebted to Dr. Darab Dastur for valuable suggestions and constructive criticism. Thanks are due to Dr. Herbert M. Katzin and Dr. A. Dellaporta, who put at our disposal some of the human eyes used in this study.

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Discussion

Dr. Peter Kronfeld (Chicago): The findings of Dr. Holland and associates are of interest both from the standpoint of morphology and of physiology.

As the essayists have stated, a recent paper by the Czech author, Vrabec, caused quite a stir about two years ago. Until that time very little was known about the innervation of the chamber angle. Dr. Vrabec's paper undoubtedly contained a great deal of new and valuable information but, at the same time, that paper left a good many questions unanswered.

The punch line of Dr. Vrabec's paper was the demonstration of a lamellated body in a trabecular zone of the goose, Ophthalmologica (**128**:359, 1954).

When I first studied the microphotograph, it looked absolutely spectacular to me.

The structure described is very similar to the well-known Pacinian bodies and is practically identical with the finding of Herbst in the mucous membrane of the tongue of the duck.

Obviously, Dr. Vrabec was aware of the significance of this finding and stated, at the conclusion of his paper, that he would make a special search for specific nerve endings in the trabecular zone and in the ciliary body of various vertebrate eyes. Two years have passed without further words from Dr. Vrabec.

The systematic studies by Dr. Holland and associates show clearly that organized end-organs, end-organs with a specific structure, are not a regular occurrence in the trabecular zone of any of the animals which we use for the study of aqueous dynamics or of regulation of the intraocular pressure. We can be reasonably sure that in the eye of the monkey, the rabbit, and the cat, structures such as encapsulated end-organs do not exist. That is unfortunate because encapsulated bodies or specifically end-organs make the study of innervation very much easier. Without such help today's essayists have done very well. They have presented convincing evidence that free terminations of nerve fibers are a regular, constant finding in the trabecular zone of the human eye as well as that of the rabbit and the monkey.

I am in complete agreement with the authors in that no conclusions should be drawn with regard to the nature of those fibers, and it might be best at this point to say that, in all probability, these fibers are not all of the same nature. But to those of us who are primarily interested in trying to find out how the very interesting phenomena, regulatory with regard to intraocular pressure, which have been discovered in the last four or five years, come about. The findings of Dr. Holland are most welcome, irrespective of what the nature of those fibers is. I am certainly looking forward to the results of degeneration experiments.

There is one question I would like to ask Dr. Holland. Do free nerve endings of the type demonstrated here today in the trabecular zone, occur in any other portion of the sclera? I am not referring to the cornea. Do they occur in the region of the lamina cribrosa?

I think it would be important to know that such

fibers and such terminations are a very rare finding anywhere else in the sclera.

Dr. Holland and Dr. von Sallmann have given us a good, solid, new piece of knowledge to be incorporated in the annals of ophthalmology.

DR. MONTE HOLLAND (closing): I should like to thank the discussers for their very enlightening remarks, and for pointing out some of the things that Vrabec has done.

I want to mention that in his study serial sections were not mentioned, and in a study of this type it is almost a sine qua non, because one very often finds various types of artefacts which can easily be confused with organized nerve terminals. We have come across structures which superficially resembled organized endings which on serial section proved to be fragments of either blood vessels or nerve trunks cut in different planes.

I also want to point out that no one technique is sufficient for the study of neurohistologic details, and that the use of more specific methods, such as methylene blue vital staining, has added immeasurably to our understanding of the innervation of this region.

As to the endings in other places—this type of ending is observed in the iris and ciliary body and, as mentioned in the paper, in the cornea. This is not an unusual finding; it is very common. In other areas innervated by the autonomic nervous system, the same type of terminal is found.

One of the main purposes of this paper was to show that organized endings are not necessary at all in order to postulate that nervous receptors can act in the regulation of the intraocular pressure. As pointed out, in other pressure receptor areas which are well known, there are only free nerve endings similar to the type we have described.

A TECHNIQUE FOR THE DETERMINATION OF BLOOD-VOLUME CHANGES*

IN THE CHOROID AND RETINA BY THE USE OF RADIOACTIVE PHOSPHORUS

JEROME W. BETTMAN, M.D., AND VICTOR G. FELLOWS, JR., M.D.
San Francisco, California

For several decades numerous drugs which have been presumed to have a vasodilating effect upon the vessels of the retina and choroid have been used to treat conditions presumed to be due to a diminished blood supply. In many instances the effectiveness of these drugs has been open to considerable question.

We, as well as many other authors, have used radioactive phosphorus as a diagnostic aid to determine whether or not a fundus lesion was malignant.[1-3]

It was soon discovered that the radioactivity of a lesion being tested for potential malignancy often depended more upon the vascularity of the tissue than any other factor. This was especially true if the count was done within the first few hours after the injection of radioactive phosphorus. This suggested to the present writers that radioactive phosphorus might be more useful as an index of the vascularity of the tissue, and the changes in that vascularity, than it has been as a diagnostic aid in the detection of intraocular tumors.

There have been many other experimental attempts to determine the effect of vasodilators on the blood vessels of the retina and of the choroid. None of these methods has been completely successful. These techniques of determining effects of vasodilators can be grouped into:

A. Those dependent upon direct observation of the vessels of the retina and the choroid.

B. Those dependent upon secondary physiologic effects resulting from the changes in vascularity.

* From the Department of Surgery, Division of Ophthalmology, Stanford University Medical School. This study was supported by a Grant-in-Aid from the U. S. Department of Public Health, Education, and Welfare. Presented before the Association for Research in Ophthalmology (Western Section), February 7, 1956, San Francisco, and at the Oregon Academy of Ophthalmology, November, 1955.

A. Direct observation of the blood vessels

This technique includes: (1) Observation of the retinal vessels with an ophthalmoscope, with or without a grid;[4] (2) photography with the Nordenson camera;[5] (3) photography with a microscope and Koeppe contact lens;[6,7] (4) the scleral window technique for the observation of choroidal vessels according to the method of Leopold.[8]

The first method, that of direct observation of the retinal vessels with the ophthalmoscope, has resulted in totally unsatisfactory results. It is difficult, if not impossible, to maintain fixation upon a given segment of a vessel for a long period of time. The changes in the vessels are often too subtle to be detected by direct observation. The changes resulting from varying degrees of astigmatism and of aberrations in different sections of the cornea produce results which may be more marked than those resulting from changes in caliber of the blood vessels. This method, as a whole, is quite unsatisfactory, but even if it were effective it would only indicate changes in the larger retinal vessels and would indicate little as to the total blood volume of the retina and the choroid.

Studies of the caliber of the retinal blood vessels with a Nordenson camera are likewise less satisfactory than one would expect[6,7] This method is unsatisfactory because: (a) The magnification is limited to six times; (b) the phase of the pulse influences the size of the blood vessels; (c) the apparent size of the blood vessels varies with the state of focus; (d) the retina is not a flat field, and therefore distortion is present at the periphery; and (e) the motion of the animal interferes with observation. In addition this method, likewise, only indicates the status of the larger retinal vessels.

Photography of the retinal blood vessels using the Leitz Ultropak microscope and a Koeppe contact lens results in more magnification than that obtained with the Nordenson camera, but the other limitations described in association with the Nordenson camera are also present with this method.

The interesting and ingenious scleral window technique of Leopold provides a method to study the choroidal circulation.[8] This technique involves trephining a window in the sclera, in an experimental animal, and observing the choroidal vessels through this trephined area using a microscope. This method only lends itself to the study of the larger choroidal vessels, and, of necessity, physiologic relationships may well be altered as the result of the trauma produced by the creation of the scleral window.

B. Techniques dependent upon secondary physiologic effects resulting from the changes in vascularity

Other attempts have been made to study vasodilators by evaluating physiologic changes presumed to be secondary to alterations in the blood supply. These methods include changes in the electroretinogram by Henkes,[9] studies of the flicker fusion frequency by Scher and Spankus,[10] and studies of angioscotometry.

Henkes confirmed Karpe's[11] original investigation that circulatory disturbances in the retina produced variations in the electrical response. Although it may be true that the shape of the electroretinogram depends upon alterations in the circulation of blood, the actual use of this technique as a method of demonstrating the effect of vasodilator drugs is open to question. Henkes reported on two cases in one of which acetylcholine was given retrobulbarly in the abnormal eye and in the opposite normal eye. The effect was the same in both eyes. He rarely uses the electroretinogram for this purpose because of the inconsistent results.[12] Hellstrom[13] studied the effect of anoxia in kittens and found that changes in the electroretinogram were not constant. Although the electroretinogram may be an important indicator for studying the action of vasodilator drugs, the reliability of this technique

has not yet been established. Jacobson and Lincoln[14] used the electroretinogram potential in the study of various drugs presumed to have a vasodilating effect. The results were variable, and made the evaluation of these drugs difficult indeed.

The flicker fusion frequency has been used by Scher and Spankus[10] to evaluate possible vasodilators. The results by the use of this technique were not striking. Hypoxia produced no striking change, although rebreathing into a bag did reduce the fusion threshold after two minutes.

Angioscotometry has also been used to evaluate the effect of agents upon the status of the retinal vessels. The effect, if any, is a secondary one and provides an indirect measure of the retinal blood vessels, and only the retinal vessels.

The correlation of vascular events within the eye with changes in the intraocular pressure is very difficult to achieve since many other factors must be eliminated.

Use of radioactive phosphorus

When radioactive phosphorus in the form of sodium acid phosphate is injected intravenously the phosphorus finds its way into the vascular tissues of the eyeball almost to the exclusion of the nonvascular tissue, as may be seen in Table 1.

As may be seen from Table 1 soon after the injection of P^{32} almost all of the radioactivity comes from such vascular tissues as the external ocular muscles, the choroid and retina, and the ciliary body. The interior of the eye, the lens, aqueous, vitreous, as well as the sclera and cornea, are almost free of radioactivity. If the sodium acid phosphate is injected directly into the blood vessels, the P^{32} soon diffuses out of the vascular tissues and into the aqueous, vitreous, and other structures of the eye. If, however, the radioactive phosphorus is incubated with the red blood cells, previously withdrawn from a vein, and then reinjected, after the method of Berson and Yalow,[15] the P^{32} and hence the radioactivity is confined to the vascular tree and does not diffuse out into the tissues. We have demonstrated repeatedly that the radioactivity of the blood remains almost constant for a period of more than an hour and a half by the use of this technique. This is shown in Figure 1.

TABLE 1

Distribution of P^{32} in various ocular structures

Material	Activity in Counts per Second per gm. of Tissue
External ocular muscle	180.0
Choroid and retina	86.0
Ciliary body	72.0
Cornea	27.0
Lens capsule and cortex	15.8
Aqueous	6.0
Sclera	2.0
Vitreous	1.0
Lens nucleus	0.9

The details of this technique are as follows: 10 cc. of blood are withdrawn from the experimental animal, heparinized, and mixed with 2.0 mC. of radioactive phosphorus. The mixture is incubated in a rubber stoppered centrifuge tube at 37°C. for two hours, and during this time it is gently agitated. The plasma and buffy coat are removed, and the labeled red blood cells are then centrifuged and washed three times

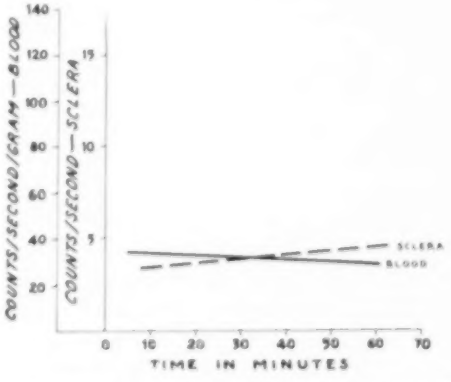

Fig. 1 (Bettman and Fellows). Radioactivity of the blood remains constant for more than an hour and a half.

with isotonic saline. The third washing is sufficient to reduce the radioactivity outside of the red cells to approximately one percent of the total. The radioactive, resuspended red blood cells are then reinjected into the experimental animal.

Cats were used in all except the early experiments because: (1) There is a great similarity between the retinal circulation of the cat and of man;[16] (2) the reflexes of the cat are similar to man; and (3) in some respects they are easier to work with than a number of other experimental animals.

The animals were anesthetized with pentobarbital (Nembutal) intraperitoneally at a dosage of 30 mg. per kg. of body weight. Curare was injected to assure smooth respirations while the animals were being respired artificially.

Because all of the radioactivity is now confined to the red blood cell mass and because the red blood cells must be entirely within the blood vessels, and the blood vessels are almost all within the choroid and retina in the posterior segment of the eye, we now have a method for the determination of the blood volume in these structures.

It is essential to use P^{32} rather than some other radioactive isotope because P^{32} is a pure beta emitter. The energy of the beta particles given off by the P^{32} is such that they penetrate through an average of three mm. of tissue, a maximum of eight mm. This energy is sufficient to penetrate through the sclera, but not sufficient so that beta particles arising outside the eyeball can gain access into the counting tube. Reference to Figure 2 may clarify this.

A Geiger tube held over the sclera after the injection of P^{32} receives impulses from the sclera, the retina, the choroid, and the vitreous. It cannot receive impulses from tissue further removed because the beta particles do not have sufficient energy to travel so far. Of the four tissues mentioned only the choroid and retina have a well-developed blood supply and consequently the blood volume is largely contained within

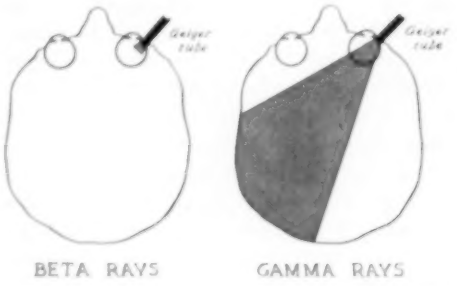

Fig. 2 (Bettman and Fellows). Comparison of gamma and beta rays.

these tissues. If a radioisotope emitting some gamma rays were used, the response would be obtained from the entire head of the animal as shown in Figure 2, and one can evaluate nothing as to the status of the blood vessels within the eye itself.

By the use of this technique we felt that we were in a position to evaluate the effect of any of the presumed vasodilators upon the vessels of the retina and the choroid.

The efficiency of this method was demonstrated early in the project because of the marked inconsistency of the counts of radioactivity obtained over the sclera. We were unable to account for this lack of consistency until it was realized that the respirations of the animal were not controlled, and that carbon dioxide, one of the best vasodilators, could well be accumulating within the animal without our knowledge.

Consequently subsequent animals were tracheotomized, a tube was inserted into the trachea, and a breathing machine connected to the animal so that a given amount of air was supplied, respirations were controlled, and the carbon dioxide was not allowed to accumulate. When this modification was adopted, the results became perfectly consistent. The effect of carbon dioxide upon the volume of blood in the choroid and retina can be seen from Figure 3.

This technique gives consistent counts over the sclera when the CO_2 is controlled. The radioactivity within the blood stream is likewise constant, as demonstrated in Figure 1.

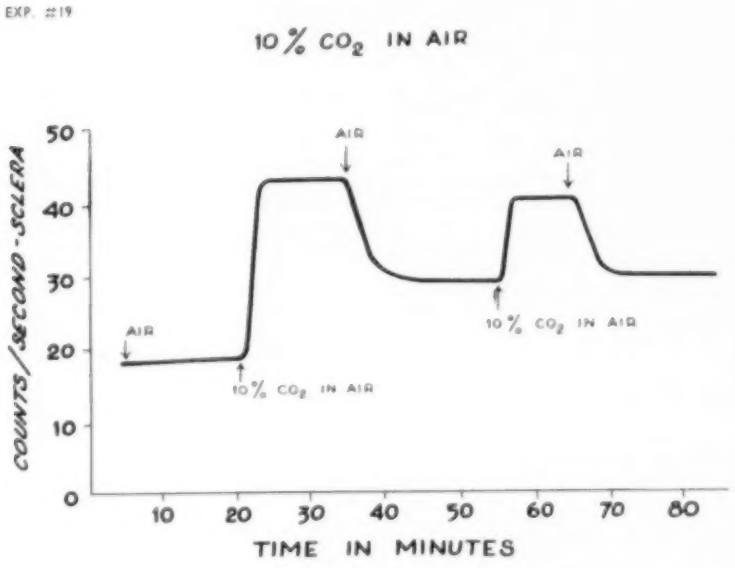

Fig. 3 (Bettman and Fellows). The effect of carbon dioxide upon the volume of blood in the choroid and retina.

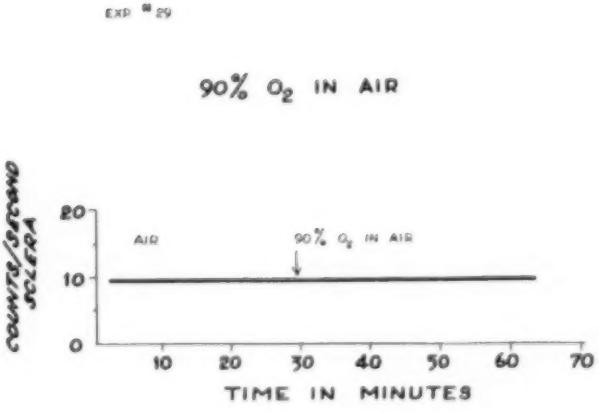

Fig. 4 (Bettman and Fellows). Alterations of oxygen concentration resulted in no blood-volume change in the choroid and retina.

Altering the CO_2 concentration produces marked changes in the blood volume of the choroid and retina as demonstrated in Figure 3.

Alterations in O_2 concentration resulted in no change in the blood volume of the choroid and retina as shown in Figure 4.

A systematic study of all agents likely to cause changes in the retinal-choroidal blood volume is being conducted using the technique described. The results will be reported in a subsequent paper.

2400 Clay Street (15).

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Discussion

Dr. A. Edward Maumenee (Baltimore): The authors have limited their discussion to a description of a technique for determining the change in blood volume in the choroid and retina. This technique appears to me to be a milestone in the study of physiology of the eye, because it produces so little disturbance in the structures that are to be tested.

I think that the value of this test will depend on two factors. First, the sensitivity of the test, that is, if satisfactory minor variations in the change of the blood volume can be detected and whether the results obtained can be repeated from day to day. Second, the toxicity of the radioactive elements injected. If this latter factor is not a problem, then red blood cells of various blood types could be tagged and preserved by the freezing technique. Thus, material would be available for repeated testings of the same patients or for testing a patient at a moment's notice.

The authors have not discussed many of the questions which I am sure they plan to investigate in the future. Thus, they have not attempted to differentiate between the retinal and choroidal circulation or between the circulation of the larger vessels in the choroid and the choriocapillaris. The retinal circulation could be eliminated by clamping the optic nerve or by direct obliteration of the central retinal artery. The fact that Leopold failed to note any dilatation of the larger choroidal vessels after carbon dioxide administration, and the authors have noticed marked increase in their radioactive count following the administration of carbon dioxide, suggests that this increase in blood volume is due to a dilatation of the choriocapillaris vessels.

The technique described could probably be applied to many problems of ocular pathology. Thus, counts taken in patients during an attack of acute congestive glaucoma and repeated after the attack has been brought under control, would aid in determining whether congestion of the choroidal vessels was a factor in acute congestive glaucoma. Other pathologic conditions which might be studied would be retinitis pigmentosa and various other vascular lesions in the fundus and choroid.

Dr. Bettman and Dr. Fellows are to be congratulated on the development of this ingenuous technique for the study of the perplexing problems of choroidal and retinal circulation.

Dr. Ludwig von Sallmann (Bethesda, Maryland): I am sorry to disagree with the practical applicability of studying blood flow in the anterior part of the eye using radiophosphorus tagged red cells. In the Ophthalmology Branch of the National Institutes of Health this technique of labelling the red cells was used according to the method of Bergson and Yallow. Two or three properly curved counters were placed in close proximity to the limbus area of cats and were connected with a scaler. The latter was connected with a rate meter and,

for continuous recording, with an Esterline-Angus milliameter. No changes of the beta particle emission were observed during electric stimulation of the hypothalamus at times when intraocular pressure rose steeply. From previous experiences with temperature measurements in the subscleral space it was known that this rise is caused by an increase in blood flow. On the basis of these observations I doubt that the method under discussion really will provide a valuable tool for judging the effects of vasodilators on intraocular vessels or for studying conditions of the intraocular vasculature by this procedure.

Dr. Victor G. Fellows, Jr. (closing): I would like to thank Dr. Maumenee and Dr. von Sallmann for discussing our paper. In regard to Dr. von Sallmann's remarks it should be pointed out that in his work the radioactivity was not measured over the choroid and retina as described in our technique but over the ciliary body instead. Also he did not administer carbon dioxide to his animals and then measure the change in radioactivity. I also understand that Dr. von Sallmann's Geiger-Müller tube differed considerably from the type used by the authors. Because of the above differences in the experimental set-up one would not expect Dr. von Sallmann's and our conclusions as to the efficacy of the method we have described necessarily to agree.

In conclusion I would like to emphasize that our method does not cause marked physiologic disturbances and that the results obtained are consistent and statistically significant. A full review of our findings will be reported in a subsequent paper.

CATIONS AND THE CORNEA*

Toxicity of metals to the stroma

W. Morton Grant, M.D., and Harold L. Kern, Sc.D.
Boston, Massachusetts

Foreword

The studies to be reported here represent a single phase of our interests in the interactions of ions with the corneal stroma. From the physiologic standpoint, we have been intrigued with the implications of binding of ions by the stroma in relation to the osmotic gradients and turgescence of the cornea, and from the toxicologic standpoint we have been concerned with the influences of foreign ions on the chemical, physical, and vital properties of the cornea. Our experimental work has so far been related principally to the actions of cations. Currently we are examining the physiologic relationship of ions such as sodium in the cornea and the toxicity to the cornea of organic cations such as quaternary ammonium surfactants and certain dyes. Our most extensive studies, however, have been on the toxic effects of metal cations, and it is this phase of our work which we are to report here.

Introduction

In this investigation we have examined systematically the toxicity of salts of the metallic elements to the corneal stroma, and have analyzed certain of the chemical reactions of cations involved in corneal injury. We have also sought to reverse these reactions for therapeutic purposes. Although our investigations have been prompted by specialized interests in ophthalmic toxicology, we believe that the results obtained may have significance outside of ophthalmology and toxicology since the reactions of the corneal stroma may well be representative of those of connective tissues in general.

Experiments and Results

The investigation to be reported here consists of the following parts:

1. Evaluation of injuriousness of metal cations to the corneal stroma in rabbit eyes.
2. Determination of the chemical "binding" of these cations by the stroma.
3. Measurement of "denaturing" effects on stromal structure.

* From the Howe Laboratory of Ophthalmology, Harvard Medical School, and the Massachusetts Eye and Ear Infirmary. This investigation was supported by research grant (B-103 (C3)) from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, United States Public Health Service.

4. Attempts at therapeutic reversal of binding, denaturation, and injury by means of special antidotes.

The aspects of this work which are primarily ophthalmologic will be presented in this report; the details of chemical studies are to be published separately by Kern and Grant in a chemical journal.

1. Evaluation of toxicity to rabbit eyes

An estimate of the injuriousness of metal cations to the corneal stroma at or near neutrality was obtained by dropping an approximately neutral (pH 5.0 to 7.0) 0.1 M solution of a salt of each metal on the scraped cornea of one or more rabbit eyes for 10 minutes. The chloride salts of the metals were generally employed, but in a few instances nitrate or sulfate salts had to be used because of solubility requirements. When the salts formed acidic solutions these were brought into the range of pH 5.0 to 7.0 by addition of ammonia. Addition of ammonia was necessary in only a small proportion of the metals, and the amount needed to reach pH 5.0 was generally so small that metal ammonium complex formation probably did not occur to a significant extent. Prior to exposure of the eyes to these solutions the rabbits were anesthetized by means of intravenous Nembutal, and the corneal epithelium was removed by scraping with a scalpel. After exposure, the degree and type of injury was evaluated by periodic examination during several months. In control eyes removal of the epithelium and exposure to water or a neutral solution of ammonium chloride caused no permanent damage.

The results of tests on rabbit eyes are summarized in Table 1, which presents the cationic portion of the periodic chart of the elements. This chart has been annotated with our experimental findings on toxicity to the corneal stroma. The severity of corneal opacification has been indicated beneath the symbol for each of the elements tested. This is expressed in terms ranging from 0 to 4-

TABLE 1

Periodic chart of the metallic elements, with the toxicity of the salts of the metals to scraped rabbit corneas indicated in units ranging from 0 (for no permanent injury) to ++++ (for complete permanent opacity). Elements having salts which were insufficiently soluble to test at 0.1 M concentration at pH 5 to 7 have been crossed out, or if tested at slightly below this concentration have results enclosed in brackets

I A	II A	III B	IV B	V B	VI B	VII B	VIII	I B	II B	III A	IV A	V A	VI A
H													
Li 0	Be (++++)												
Na 0	Mg 0									Al			
K 0	Ca 0	Sc (++++)	Ti	V	Cr	Mn +++	Fe	Co +++	Ni +	Cu ++++	Zn	Ga	Ge
Rb 0	Sr 0	Y ++++	Zr	Nb	Mo	Te	Ru	Rh +	Pd	Ag ++++	Cd ++++	In	Sn	Sb
Cs 0	Ba 0	La ++++	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg ++++	Tl	Pb ++++	Bi	Po

RARE EARTHS	Ce (++++)	Pr ++++	Nd ++++	Pm	Sm ++++	Eu	Gd ++++	Tb	Dy	Ho	Er	Tm	Yb	Lu

plus; 0 representing no permanent injury and 4-plus representing complete permanent opacification. Some of the elements could not be tested under the standard conditions described in the experimental section because of inadequate solubility in the range of pH 5.0 to 7.0; those which were omitted for this reason have been crossed out in Table 1. Certain other elements were not tested because they were too rare or too costly. In the case of beryllium, scandium, cerium, and lead, solubility was insufficient to provide a 0.1 M solution, but when tests were carried out employing more dilute solutions these metals were found to be so toxic to the cornea even in the more dilute solutions that the results of the tests have been included in the table. The results for these elements are enclosed in brackets in the table to indicate nonstandard conditions.

With the notable exception of beryllium, the cations of the first two groups (IA, IIA) of the periodic table (lithium, sodium, potassium, rubidium, caesium, beryllium, magnesium, calcium, strontium, and barium) were found to cause no permanent damage to the cornea. The corneas recovered promptly following exposure to the salts of all of these elements except beryllium, and recovery was essentially the same as for control corneas exposed only to water or 0.9-percent sodium-chloride solution. However, following exposure to beryllium (approximately 0.05 M sulfate at pH 5.0) the corneas developed diffuse gray opacity after a latent period of several hours. This opacification increased slowly from the first day to several weeks after exposure, the corneas eventually becoming completely opaque and necrotic. The appearance at six days following exposure is shown in Figure 1.

In contrast to groups IA and IIA, all of the elements in the third group (IIIB) including scandium, yttrium, and the rare earths (for example, lanthanum, cerium, praseodymium, neodymium, samarium, and gadolinium) caused total and permanent opacity of the cornea (see figs. 2 to 9). Exposure of corneas to the elements of this group of metals induced a characteristic sequence of changes. The corneas generally remained clear during the actual exposure to neutral solutions of the metal salts, but after a latent period of several hours diffuse gray opacities gradually developed. Subsequently, over a period of two to three weeks, these

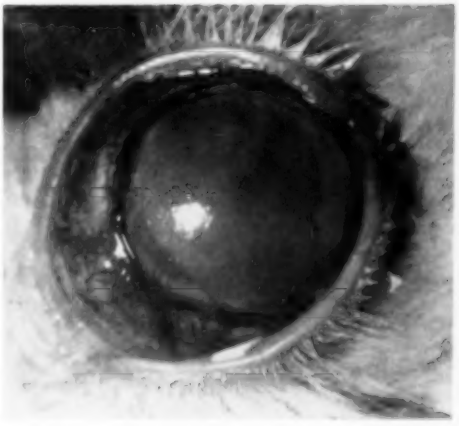

Fig. 1 (Grant and Kern). Beryllium induced opacity of rabbit cornea six days after exposure to approximately 0.05 M solution of the sulfate at pH 5 for 10 minutes.

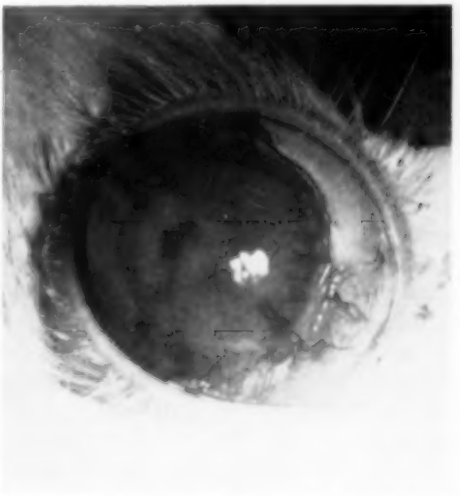

Fig. 2 (Grant and Kern). Scandium induced opacity of rabbit cornea 17 days after exposure.

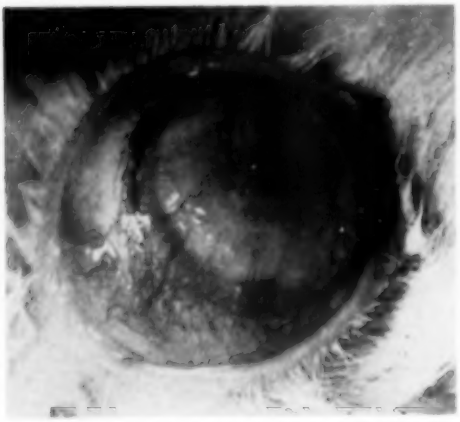

Fig. 3 (Grant and Kern). Yttrium induced opacity and descemetocele 17 days after exposure.

Fig. 4 (Grant and Kern). Lanthanum induced opacity seven days after exposure.

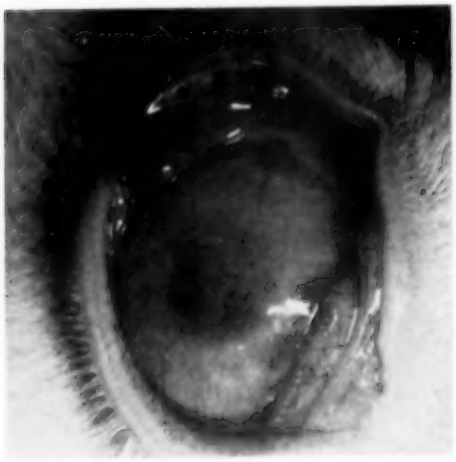

Fig. 5 (Grant and Kern). Cerium induced opacity 19 days after exposure.

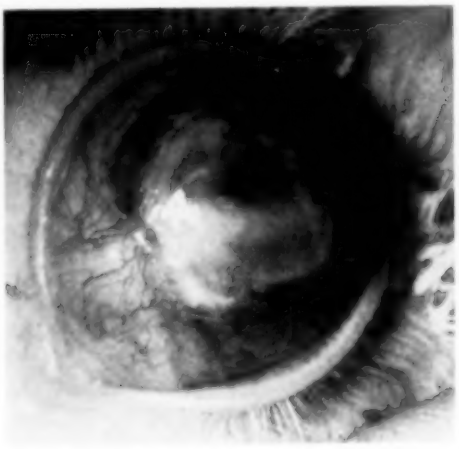

Fig. 6 (Grant and Kern). Praseodymium induced opacity 12 days after exposure.

opacities changed to dense yellowish-white plaques accompanied by extensive vascularization. In some cases the axial portion of the corneas became necrotic and some of the tissue was sloughed, resulting in spontaneous perforation.

Analysis for calcium was performed on some of the corneas which had developed dense whitish opacities during the first and second weeks following exposure to neutral solutions of lanthanum, samarium, and gadolinium,* and the results suggest that some of the opacity present in the cornea at this period after exposure to elements of group IIIB may be associated with secondary cal-

* By titration of acid extracts of these corneas with ethylenediamine tetracetate the calcium contents were found to be respectively 3.9, 3.1, and 2.6 millimoles per gm. (dry weight) of cornea, compared to a normal value of 0.04 millimole per gm.

Fig. 7 (Grant and Kern). Neodymium induced opacity six days after exposure.

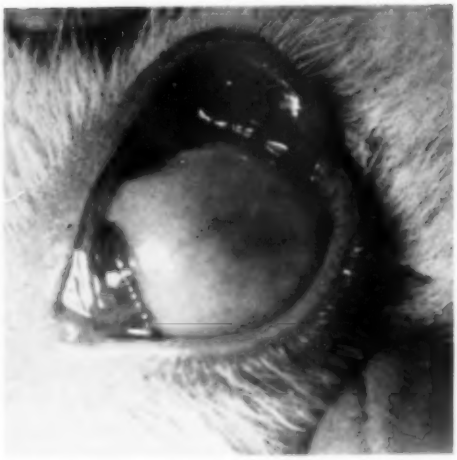

Fig. 8 (Grant and Kern). Samarium induced opacity six days after exposure.

cification. (Incidentally, the rabbit cornea seems in general to have a much greater tendency to secondary calcification following chemical injuries than does the human cornea.)

Concerning the effect of the metals of the higher groups of the periodic table which could be tested at 0.1 M concentration in the range pH 5.0 to 7.0, no special features were noteworthy except rapid complete

Fig. 9 (Grant and Kern). Gadolinium induced opacity six days after exposure.

blackening of the cornea in the case of silver. Certain of the other metals which did not have sufficiently soluble salts to test in 0.1 M solution were tested in the form of neutral suspensions of their chloride salts, having the equivalent of 0.1 mole of material present per liter. The results are not quantitatively comparable to those of the more standardized tests, and are therefore not given in Table 1, but some of the effects were interesting. Thus, ruthenium and osmium made the corneas jet black, and in the case of osmium this blackness persisted indefinitely (see fig. 10). In the case of ruthenium the initial blackening gradually disappeared, leaving a clear but yellow-stained cornea. Vanadium caused only slight yellowing of the cornea initially, but during two weeks following exposure the cornea gradually became opaque and vascularized. Lead had a strong tendency to form white plaques in the cornea.

In order to determine how much protection against injury by metal ions might be offered by the natural barrier of the corneal epithelium, certain of the elements which caused opacification of the denuded corneal stroma were tested also on eyes in which

Fig. 10 (Grant and Kern). Osmium induced persistent dense black staining of rabbit cornea, resulting from 10 minutes' application of an ammonia-neutralized three-percent osmium trichloride paste to the denuded surface.

the epithelium had been left intact. The protective value of the epithelium was strikingly demonstrated in several instances. Thus beryllium, yttrium, lanthanum, praseodymium, neodymium, and samarium caused no observable injury when applied to rabbit eyes with the epithelium intact under the same conditions of time, concentration, and pH (approximately neutral) which were associated with severe damage to the corneal stroma when the epithelium was removed.

In an effort to determine whether the gradual development of corneal opacity observed with certain metals might be secondary to derangement of a vital process in the cornea rather than the result of a direct effect of the metal on the transparency of the cornea, a comparison was made of corneas in dead and live rabbits following exposure to lanthanum, which characteristically has a latent period in vivo. Following exposure to 0.1 M lanthanum chloride, some edema but no opacity developed in the cornea of a dead rabbit during four days at room temperature, while during the same period in live rabbits definite gray opacity developed. This suggests that in the cornea of living animals opacification from lanthanum and similar metals does result from derangement of some vital process. Possibly if the initial reaction of metals with the cornea could be undone in time the visible secondary changes might be prevented.

2. Determination of chemical binding

We have studied in some detail the chemical interaction of the corneal stroma with metals and other cations, as well as the relationship of this interaction to toxicity. A full description of the experiments which have been carried out will be presented in a separate chemical publication. In brief, the binding of various cations to the corneal stroma has been examined as follows:

Excised corneas were immersed in appropriate solutions of the metal salts for study of the interaction under widely varied conditions of time, pH, and concentration. The corneas were then pressed in a hydraulic press to squeeze the solution out of the tissue, and the quantity of cation retained by the nearly anhydrous residual tissue was assayed chemically. A small correction was made for the amount of solution which was not removed by pressing, and the quantity of cation bound by the tissue itself was calculated.

In the cases of sodium, potassium, calcium, barium, strontium, and lanthanum approximately equimolar amounts were found to be bound by the tissue in contact with neutral 0.02 M solutions. However, no simple relationship was evident between the toxicity of these various metals as tested on rabbit eyes and the amount which was bound under standard conditions in vitro. The toxicities were found to differ to a much greater extent than did the quantities bound by the tissue. For instance, no appreciable corneal injury was induced by sodium, potassium, calcium, barium, or strontium, but lanthanum caused severe opacification although the number of molecules of metal bound by cornea in vitro was nearly the same in each case. Further chemical studies on excised corneas in which carboxyl groups

were blocked by methylation or by conversion to carbonamide groups indicated that the number of molecules of metal bound was governed to a greater degree by the number of anionic groups available in the tissue than by the nature of the cation. It appeared, therefore, that a relationship of cation binding to toxicity might be better sought in the quality or character of the binding than in the quantity.

In attempting to characterize the nature of the binding of various metals and to find some relationship between this and injuriousness to the cornea, we have exploited several approaches. First of these has been to examine the roles of the collagen and of the mucoproteins of the cornea in the binding of metals. This we have done by comparing binding of various metals by purified collagen, by whole cornea, and by cornea from which some of the mucoproteins have been extracted. Tested in this way, potassium and calcium were found to be bound in significantly less amount by corneal stroma from which some of the mucoprotein had been extracted than by whole corneal stroma, and still less was bound by purified collagen. It appeared from these findings that the mucoprotein is primarily responsible for the binding of potassium and calcium at neutrality. On the other hand, extraction of mucoprotein from the cornea caused only a slight decrease in binding of lanthanum, and lanthanum reacted to a greater extent with purified collagen than did either potassium or calcium. This difference, as well as other peculiarities to be described in subsequent paragraphs, suggests a possible basis for the toxicity of lanthanum in the nature of its binding.

More light has been thrown on the relationship between metal binding and toxicity by study of the interaction of cornea with mixtures of cations. This has revealed that certain cations are bound in preference to others, presumably owing to some greater affinity or strength of bond. This selectivity has been partially evaluated utilizing calcium as a standard of reference, determining the effectiveness with which various substances compete with calcium for binding to the cornea. The results have been expressed in terms of competition factors. In this scheme a numerical competition factor of one represents an affinity of a given cation for the corneal stroma equal to that of calcium; a factor of 10 represents an affinity 10 times that of calcium. These factors of course provide a measure of the affinity for only those sites in the tissue with which calcium is reactive. Competition values for several elements are listed in Table 2, along with toxicity values and additional data to be described in section 3.

Clues to the basis for differences in injuriousness of various metals may be found in the data of Table 2. When the competition factors of various metals versus calcium and their injuriousness to rabbit eyes are compared, it appears that toxicity is in many instances roughly correlated with the capability of the metals to compete for common binding sites in the cornea. For instance in the case of the metals of groups IA and IIA, all of which are non-injurious except for beryllium, none has a competition factor greater than that of innocuous calcium, except for beryllium which has an affinity for the cornea 6.5 times as great. Furthermore, in group IIIB in which all the substances are very toxic to the cornea the competition factors are many times as great as that of calcium. However, some exceptions to this correlation occur in other groups, particularly in the cases of manganese (ous) and cobalt (ous). Both of these elements have competition factors not much different from those of the innocuous metals, yet they are found to cause considerable corneal opacification.

In those cases where toxicity has been found out of proportion to competitive affinity, there is the possibility already suggested by comparison of binding of calcium and lanthanum to mucoprotein and to collagen that some of these metals may attach to

TABLE 2

INTERACTION OF METAL IONS WITH THE CORNEAL STROMA AT pH 5 TO 7

Metal	Injury to Cornea in Rabbits Range: 0 to ++++	Tendency to Bind to Cornea *Competition Factor vs. Calcium* (Calcium = 1)	Postexposure Water Absorption Capacity of Cornea (percent of normal)
Lithium	0	0.07	100.
Sodium	0	0.08	100.
Potassium	0	0.06	100.
Rubidium	0	0.09	100.
Caesium	0	0.07	100.
Beryllium	++++	6.5*	20.
Magnesium	0	0.8	100.
Calcium	0	1.0	100.
Strontium	0	1.0	100.
Barium	0	0.5	100.
Yttrium	++++	96.	10.
Lanthanum	++++	70.*	10.
Praseodymium	++++	77.	10.
Neodymium	++++	61.	10.
Samarium	++++	108.	—
Gadolinium	++++	79.	—
Thorium (pH 4.5)	++	145.	80.
Manganese (ous)	+++	0.2*	100.
Cobalt (ous)	+++	1.5	95.
Nickel (ous)	+	1.6	55.
Copper (ic)	++++	18.5	10.
Silver	++++	4.	10.
Cadmium	++++	2.5*	20.
Mercury (ic)	++++	—	10.

* = In addition some binding which is noncompetitive with calcium has been found in these cases.

special sites in the cornea which are not reactive with calcium or other nontoxic metals. This special binding would not be revealed by measurements of calcium displacement, and, conversely, binding of calcium or other innocuous cations by the cornea would not be expected to prevent binding at sites which do not react with calcium. We have investigated this possibility by determining whether the binding and injuriousness of certain of the toxic metals could be prevented by addition of a high concentration of innocuous cations. Indications have been obtained in this manner that at least manganese, lanthanum, and beryllium do bind in part to sites in the tissue immune from competition with calcium and potassium. It seems from the evidence obtained so far that toxicity in many instances is related to the capability of the metals to compete for common binding sites in the cornea, but in some instances (for example, manganese, beryllium, lanthanum) toxicity may be associated with binding to special sites. We do not know as yet whether toxicity is a function of bond strength at these special sites, nor have we identified these sites in the tissue. However, in the case of lanthanum we have a clue noted earlier in the exceptional binding by the collagenous moiety of the cornea.

If toxicity were related to strength of bond between metal and cornea, it might be expected that toxic metals would persist in the cornea in vivo longer than innocuous metals. We have obtained results consistent with this supposition in comparing the persistence of calcium, yttrium, and lanthanum in the corneas of rabbits following exposure to neutral 0.1 M solutions of the chlorides of these metals. Calcium was found to disappear rapidly, approaching the physiologic concentration within two to three hours, whereas yttrium and lanthanum persisted in the cornea with slow decrease in concentration during several weeks. This suggests the possibility that continued presence of foreign

metal molecules bound to the cornea may have adverse effect, as well as the possibility that removal of these molecules may have therapeutic advantages.

3. Measurement of denaturing influence of metal binding

In attempting to explain why the binding of cations by the cornea has injurious influence, whether in relation to strength of bond or to peculiarity of reaction site, we suppose that the interaction alters physicochemical properties of the tissue, and this in some manner interferes with normal functions. Whether this supposed alteration of properties is induced only while bound metal is present and can be corrected by removal of the metal, or whether it is irreversible once induced, is of course most significant from the standpoint of treatment. Our first step in examining this situation has been to investigate the influence of cation binding on certain of the physicochemical attributes of the cornea. Attempts at treatment will be described later.

Evidence of physicochemical alteration of the corneal stroma by the action of metal salts has been obtained in two ways: by measurement of effect on the capacity of the cornea to absorb water, and by evaluating the effect on the solubility of the corneal mucoprotein. As the basis for these measurements, it is well known that excised normal cornea placed in water swells to about 10 times its ordinary weight. Mucoproteins equivalent to about 14 percent of the dry-weight of the cornea are dissolved out under the same conditions. We have evaluated the effect of metals on corneal turgescibility and mucoprotein content by measuring the change in wet-weight and in dry-weight which occurred when pieces of cattle cornea were allowed to swell in water for two or more days following exposure for one hour to 0.1 M solutions of the metal salts (at pH 5.0 to 7.0). After exposure to the metal salts the pieces of cornea were thoroughly rinsed with water, and the water in which they were allowed to soak was changed several times so that uniform conditions (essentially uncontaminated water) were provided for comparing swellings.

The results are listed in Table 2 along with data on binding of the metals by cornea and data on injuriousness to rabbit eyes for comparison. It is evident from the information in this table that the degree to which various metals inhibit the turgescibility of the cornea generally parallels the affinity of binding of these metals to the cornea and the capacity to cause injury in the living eye. Exceptions are notable in the cases of manganese and cobalt.

The influence of metals on the solubility of mucoproteins of the cornea, as another measure of possible denaturing action of metals, has been estimated by comparing the dry-weights of pieces of cornea before and after the metal-exposure and water-soaking procedures described in the foregoing paragraph. For this estimation the initial dry-weight was calculated from the initial wet-weight, and the final dry-weight was determined directly by drying and weighing. In some instances, assay for ester sulfate was employed in determining more specifically the extractability of sulfated mucopolysaccharides. Exposure of cornea to innocuous metal salts generally caused little or no interference in subsequent extraction of mucoprotein by water, but after exposure to the toxic metal salts the extractability of mucoprotein was significantly impeded in most instances. The strongest interference was caused by yttrium, lanthanum, praseodymium, thorium, copper, silver, mercury, lead, and beryllium. Sulfated mucopolysaccharides specifically were found to be rendered almost completely inextractable in water following exposure to lanthanum chloride.

It is interesting to correlate these findings with the data of Table 2. It appears that interference with extractability of mucoprotein by various metals closely parallels inhibition of water absorption by the cornea,

and with certain exceptions these effects both parallel toxicity as manifested in living rabbit eyes. Manganese and cobalt react in an exceptional manner, in that they cause considerable injury to the eyes of rabbits, yet they scarcely interfere with corneal turgescence or with extractability of mucoprotein in water. An additional anomaly for these two metals to which attention was called earlier is a disproportion between toxicity and tendency of the metal to bind to the cornea as measured by competition with calcium. As one possible explanation of such exceptional properties the probability has been discussed in section 2 that manganese reacts with the cornea at some special site.

On the other hand, the possibility has not been eliminated that the valence of manganese and cobalt may be raised (from two to three) in the living cornea with possible attendant increase in toxicity, and that this may not occur in corneas in vitro. Obviously there is need to investigate further this apparent exception to what otherwise appears as a significant general correlation between toxicity, disturbance of hydration properties, and interference with mucoprotein extractability as a result of cation binding by the cornea.

4. Therapeutic reversal

Since we have obtained indications that the injurious effects of metals on the corneal stroma may in general be related to tenacious binding of the metals by the stroma, it has seemed logical to attempt to undo the influence of the metal and to reverse the injurious effects by removing the metal from its combination with the stroma. In seeking to accomplish this we have examined the effects of several agents which might be expected to remove metals from combination with tissue.

As one measure of effect we have determined the ability of these agents to restore the capacity of the cornea to absorb water after exposure to toxic metal salts, and in certain instances we have evaluated the influence of the potential therapeutic agents in living rabbit eyes in preventing corneal opacification and scarring. So far, we have sought to undo the effects of toxic metals principally through application of the chelating agent ethylenediamine tetraacetate (EDTA), which has great affinity for many metals and which one of us previously found effective for treating superficial corneal calcification.[1, 2] In the case of beryllium we have also examined the potential therapeutic application of ammonium aurin tricarboxylate and of sodium sulfosalicylate. Some observations have also been made on the influence of EDTA on persistence of yttrium in the cornea.

The influence of ethylenediamine tetraacetate on the capacity of the cornea to absorb water after exposure to metal salts was examined by placing weighed pieces of cornea in 0.1 M solutions of the metal salts for one hour, followed by 0.1 M neutral sodium EDTA for one hour, and prolonged soaking in distilled water in the same manner as described in section 3. The weights were determined, and the water absorption was calculated. In testing the other potential therapeutic agents similar procedures were employed. Results are shown in Table 3 under the heading of Water Absorption Capacity, and values are compared for the effects of different metals with and without treatment. It is evident that in many instances the apparent denaturation of corneal structure caused by metals is reversible, at least in so far as this is measured by turgescence properties.

The influence of treatment on development of corneal opacity following exposure to toxic metal salts was investigated in rabbits as follows. The denuded corneas of anesthetized animals were exposed to 0.1 M solutions of metal salts at pH 5.0 to 7.0 for 10 minutes in the manner described in section 1. Both eyes of each rabbit were exposed in identical fashion, and one eye was subsequently bathed with 0.9-percent sodium-chloride solution as a control, while the

TABLE 3

INTERACTION OF METAL IONS WITH THE CORNEAL STROMA AT pH 5 TO 7, AND TREATMENT BY BATHING WITH VARIOUS EXPERIMENTAL ANTIDOTES

Metal	Experimental Antidote	Water Absorption Capacity of Cornea (percent of normal)		Residual Injury in Rabbit Eyes (Range: 0 to + + + +)	
		*Control ½	Antidote ½	*Control ½	Antidote ½
Beryllium	EDTA (0.03 M)	20.	100.	+ + + +	+ + + +
Beryllium	Aurin tricarboxylate (0.1 M)	20.	70.	+ + + +	+ + + +
Beryllium	Sulfosalicylate (1. M)	20.	65.	+ + + +	+ +
Yttrium	EDTA (0.1 M)	10.	100.	+ + + +	+ +
Lanthanum	EDTA (0.1 M)	10.	100.	+ + + +	
Thorium (pH 4.5)	EDTA (0.1 M)	30.	30.	+ +	
Nickel (ous)	EDTA (0.1 M)	55.	90.	+	
Copper (ic)	EDTA (0.03 M)	10.	70.	+ + + +	+ + +
Silver	EDTA (0.1 M)	10.	20.	+ + + +	
Silver	Ferricyanide-thiosulfate	10.	80.	+ + + +	
Cadmium	EDTA (0.03 M)	20.	100.	+ + + +	+ + + +
Mercury (ic)	EDTA (0.1 M)	10.	100.	+ + + +	
Lead (sat'd soln.)	EDTA (0.03 M)	10.	90.	+ + + +	+ + +

* Control ½ = bathing with water or sodium chloride solution in same manner as for experimental antidote.

other was bathed with the experimental antidote.

Results of treatment were evaluated by periodic examination and comparison of the eyes during several weeks or months. The final results are presented in Table 3 under the heading of Residual Injury in Rabbit Eyes. The grading of corneal opacification is the same as employed in Tables 1 and 2, ranging from 0 for no permanent injury, to 4-plus for complete permanent opacification.

Results are also shown in Figures 11 and 12, showing pairs of eyes which were exposed to 0.1 M yttrium chloride, with one eye of the pair subsequently treated with EDTA and the other with sodium chloride solution. It is evident from these figures and the data of Table 3 that significant therapeutic results have been achieved in some cases.

Evidence that metal is actually removed

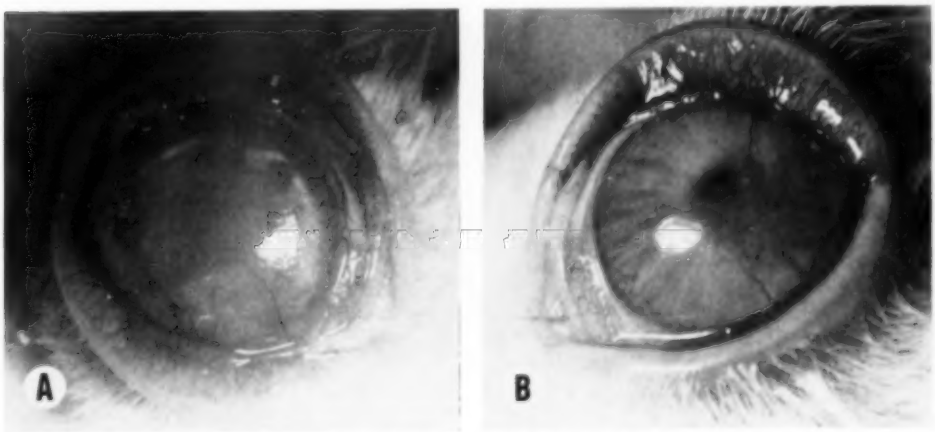

Fig. 11 (Grant and Kern). Comparison of treatments following exposure of both eyes to yttrium: (A) Irrigated with sodium chloride solution. (B) (Contralateral eye) irrigated with ethylenediamine tetraacetate solution. (Photographed 28 days after exposure and treatment.)

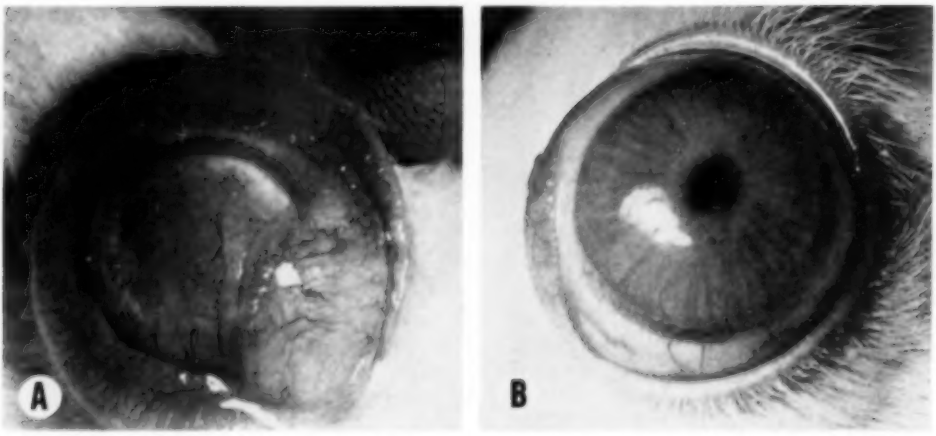

Fig. 12 (Grant and Kern). Comparison of treatments following exposure of both eyes to yttrium: (A) Irrigated with sodium chloride solution. (B) (Contralateral eye) irrigated with ethylenediamine tetraacetate solution. (Photographed 28 days after exposure and treatment.)

from the cornea by treatment with EDTA has been obtained in the case of yttrium by comparing the amount remaining in the corneas of rabbits after standard exposures to 0.1 M solution of the chloride salt with and without subsequent treatment by bathing with 0.01 M solution of sodium EDTA at pH 8.0 for 15 minutes. Assay for yttrium at various times thereafter showed that corneas treated with EDTA retained only about one quarter as much yttrium as corneas bathed in the same manner with water or physiologic salt solution.

In treatment of beryllium-exposed rabbit eyes, the therapeutic effectiveness of aurin tricarboxylate was considerably less than might have been expected from studies of its antidotal value in systemic beryllium poisoning.[8] This is presumably because of conditions and requirements peculiar to the cornea.

Bathing the cornea with 0.1 M neutral solution of the dye for 15 minutes immediately after exposure to approximately 0.05 M beryllium sulfate (pH 5.0) prevented the necrosis of the cornea which ensued in control eyes, but left the cornea stained red and opaque by the insoluble red lake formed in the beryllium-dye interaction. A similar effect was noted in experiments on cornea in vitro where it was observed that pieces exposed to beryllium and then to the dye were deeply magenta colored, presumably by the strongly colored reaction product, whereas pieces exposed to the dye alone were only faintly pink.

The formation of any strongly colored compound is, of course, undesirable in the cornea, where improvement of optical clarity is the prime objective. Compounds which form colorless soluble complexes with metals are accordingly more desirable to employ on the cornea. EDTA and sulfosalicylate have the advantage in this regard,[4, 5] and in the treatment of beryllium-exposed corneas in rabbits the results with sulfosalicylate were greatly superior to those with aurin tricarboxylate. Even better results might be expected with EDTA, since this agent is quite effective in restoring water-absorbing capacity in vitro, but in a single preliminary experiment in a rabbit the looked-for superiority was not found.

Discussion

As we have already pointed out in the foreword, the investigations which are described in this report on the interactions of

the cornea with metals have been undertaken as part of a larger study on interactions of the cornea with cations in general. The overall purpose of the study is to learn what we can of the role of cations in the physiology of the cornea, and to try to determine what goes wrong in the cornea when foreign cations are introduced and injury results. Our ultimate aim has been to gain a basis for logical development of means of treatment. An improvement in treatment is needed most urgently for alkali burns of the eye. Very little is known of the chemical mechanism in burns of this type, and, accordingly, logical means for attempting to undo the injury are lacking.

We have found in earlier studies on alkali burns that there is transient binding of the cations of the alkali to the cornea, but whether this is an important factor in initiating more complex changes in the cornea we do not yet know.[6] We hoped that we might determine how such changes are initiated by studying the interaction of cations with the cornea nearer neutrality, and the investigations reported here utilizing metal salts were undertaken in part for that purpose. In these studies, as well as in other studies currently under way on cationic surfactants and dyes, we have had the additional aim of improving the treatment for corneal injuries from these substances, although they are only occasionally encountered clinically.

These investigations have not so far provided much elucidation of mechanism of injury at high pH, but they have yielded some information which may eventually prove pertinent. They have provided a reasonable basis for development of therapeutic countermeasures for injury by metal salts and some degree of success has been achieved in the initial development of these measures. The observations which have been of most interest to the authors are that injury may be induced by interaction of the cornea with foreign cations provided the binding forces are great enough, and that in the induction of injury two phases are distinguishable. The initial phase which consists of reaction of extraneous cations with anionic groups of the tissue is more easily understandable than the later phase, since the initial reaction seems to be analogous to the well-known interaction of cations with a synthetic cation-exchange resin, exhibiting definite selectivity for different cations. We have partially evaluated this selectivity by determining how effectively various metals compete with calcium for binding by the cornea.

More difficult to analyze is the reason for the greater toxicity exhibited by substances which have high affinity for the cornea, or which in certain instances appear to bind to special groups in the cornea. High affinity and special binding suggest the existence of strong forces. Strong binding of cations might conceivably have deleterious effect either through physical distortion of tissue molecules, or through chemical interference with normal reactions of the ionic groups of the cornea. In either case, strong binding would presumably favor persistence of the metal in combination with the tissue, and it may be recalled that in rabbit eyes there was a much greater persistence of lanthanum and yttrium than of calcium.

Evidence for physical alteration of tissue by strongly or specially bound cations was found in the inhibition of swelling of cornea in distilled water after reaction with toxic metals, and in interference with extractability of mucoprotein. In the case of the polyvalent metals it might be postulated to explain these effects that individual molecules of cations could bind to anionic sites of two or more adjacent protein or mucoprotein molecules at the same time, and, if the forces involved were strong enough, could constitute a cross linkage sufficient to restrain the tissue from swelling in water or to prevent the escape of mucoprotein.

This hypothesis is weakened by the fact that swelling is also inhibited by silver, which is monovalent. Possibly neutralization of anionic charges by persistent cation binding is enough in itself to lessen the attraction

of the tissue for water. However, this may be, the interference of metals in absorption of water by the cornea constitutes an alteration of the tissue which is apparently reversible in most instances, since return to or toward normal can be achieved utilizing treatments designed to remove metals from combination with the cornea. In the few instances in which such treatment has been unsuccessful we do not yet know whether the tissue alteration was actually irreversible, or we failed to remove the metal.

After binding of metals to the cornea by bonds so strong or of such special character that physicochemical alterations are induced, another phase of reaction consisting of gradual opacification occurs. This results apparently from some disruption of vital processes in the cornea, leading to edema and abnormal dispersity of stromal constituents, as well as infiltration of cells, vascularization, and scarring of the cornea. A similar two-phase process has been recognized in the case of corneal injury by organic alkylating agents such as mustard gas; this too is characterized by an initial strong binding to the cornea which alters its physicochemical properties and leads later to opacification.[7]

Some special explanation is called for in the case of manganese and cobalt. These metals seem to provide an exception to the general correlation between toxicity, disturbance of hydration properties, and interference with mucoprotein extractability which has been found associated with binding of other metal cations to the cornea. This apparent exception is not particularly disturbing since we are already aware of the existence of other mechanisms for injury in the case of quaternary ammonium surfactants, on which we plan to report subsequently, and in injuries at high pH.[8]

From a practical as well as an academic standpoint the most gratifying findings have been that reversal of the cation binding of the initial phase of reaction can be accomplished by means of simple reagents, and that this reversal if accomplished in time can abort the opacification phase with considerable benefit in certain cases. We are optimistic that our encouraging preliminary results may be bettered through further investigation.

It seems highly probable that benefit may be obtained in human eyes contaminated with metal salts if early irrigation with sodium ethylenediamine tetraacetate is carried out. Previous clinical experience with this substance in treatment of calcific deposits in band keratopathy and in superficial lime burn of the cornea in human eyes has shown that at pH 8 it is satisfactorily tolerated at 0.01 M concentration for external irrigation of the eye,[1] or at 0.05 M when applied only to the cornea.[8] Numerous irrigations of denuded human corneas have been carried out for periods of 15 to 20 minutes utilizing 100 ml. or more of the 0.01 M solution without causing recognizable injury to the eye. More rapid and complete removal of metal compounds from the cornea might be achieved by utilizing a more concentrated solution, as was observed in experiments on rabbit eyes in the present study. However, at a concentration as strong as 0.1 M, pH 7.5 to 8.0, the solution is definitely irritating to rabbit eyes, causing edema of the stroma which lasts for several days.

It seems safer, therefore, for clinical purposes to continue to employ solutions in the 0.01 to 0.05 M range unless some clinical evidence is obtained for safety of stronger solutions. The suitability of sodium sulfosalicylate for clinical use in treatment of beryllium contamination of the cornea has not yet been ascertained, but observations on rabbits suggest that this substance probably will prove applicable in slightly alkaline solution at about 0.1 M concentration.

Summary

Systematic examination of salts of the metallic elements has shown that toxicity to the corneal stroma as measured by corneal opacification in rabbits can in most instances be correlated with tendency of the metal to

bind to the tissue, and with the denaturing influence of the metal on the cornea as measured by decrease in capacity to absorb water and by loss of solubility of corneal mucoproteins in water. The cornea exhibits a wide range of selectivity in its reaction with different metals, quite analogous to the selectivity of a synthetic cation-exchange resin. The metals which compete most effectively for attachment to the tissue are in general the most toxic. The majority of toxic metals bind to the same reactive sites in the tissue as do innocuous metals such as calcium, though with stronger affinity; certain toxic metals, however, react with the cornea at other sites.

Following the initial binding of toxic metal cations to the cornea and the generally associated alteration of hydration capacity and mucoprotein extractability, the cornea may gradually become opaque after a latent period of as much as several hours. Fortunately it is found that if the initial binding of metal to the cornea is undone, the physicochemical properties of the cornea can in some instances be restored to normal, and, if this is accomplished in time, the corneal opacification can be significantly prevented.

Most effective reversals of the influence of toxic metals on corneal hydration and mucoprotein extractability have been obtained utilizing ethylenediamine tetraacetate (sodium, pH 8.0) following exposure of the denuded corneas to salts of yttrium, copper, and lead, and by treatment with sodium sulfosalicylate following exposure to beryllium sulfate.

These results are believed to be clinically applicable, but their greater significance probably lies in what they tell us about properties of the cornea and mechanisms of injury.

243 Charles Street (14).

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THE EFFECTS OF NERVE STIMULATION ON AQUEOUS OUTFLOW IN ENUCLEATED EYES*

EDWARD W. PURNELL,† M.D., CARLTON E. MELTON,‡ M.D., AND ELLIOT Q. ADAMS, M.D.
Cleveland, Ohio

The influence of ocular innervation on intraocular pressure has been the subject of much investigation. Methods of study have included: stimulation of sympathetic and parasympathetic nerve supply, section of the autonomic nerves, and stimulation of autonomic areas in the midbrain. Stimulation of the cervical sympathetic chain in animals produces a sharp rise in intraocular pressure followed by a fall due to vasoconstriction, according to the majority of workers.[1] Section of the sympathetic innervation has given variable results.[2] According to Greaves and Perkins,[3] stimulation of the third cranial nerve in rabbits has no effect on intraocular pressure after paralysis of the extraocular muscles by decamethonium, and section of the third nerve causes no acute or chronic change in tension. Schmerl and Steinberg[4] have reported increases in intraocular pressure in cats following stimulation of the ciliary ganglion and a fall in pressure after cauterization of the ganglion.

A number of investigators have stimulated autonomic centers in the midbrain; the most extensive study has been that of von Sallmann and Lowenstein,[5] who have correlated changes in intraocular pressure with vasomotor responses, pupillary reactions, and contractions of extraocular musculature. These workers frequently noted elevations or falls in intraocular pressure which were isolated events and apparently not connected in any way with systemic blood pressure or activity of the extraocular muscles. They thought that these responses could be most plausibly explained on a local vascular basis.

Review of the past literature leaves little doubt that the autonomic innervation may influence intraocular pressure under experimental conditions. With the exception of increases in pressure due to orbital smooth muscle contraction, the responses produced have been explained in terms of alterations in the volume of the intraocular vascular bed. Singularly evident is the failure of past workers to differentiate between pressure changes in the eye due to vascular engorgement and those produced by contractions of the ciliary muscle.

The influence of autonomic innervation on aqueous humor dynamics is of more than theoretical interest, since it is especially pertinent to the yet unresolved mechanism by which autonomic drugs exert their pressure-lowering effects in chronic simple glaucoma. One theory, attributed by Duke-Elder[6] to Küsel and elaborated by Fortin,[7] postulates a decrease in resistance to aqueous outflow due to the pull of ciliary muscle fibers on the scleral spur and trabecular meshwork. A second theory concerns the influence of autonomic activity on the collector channels and aqueous veins.[8–10] There has been no direct evidence to support or invalidate these theories. No conclusions can be drawn from the changes in intraocular pressure seen in vivo during sympathetic and parasympathetic stimulation because one cannot differentiate between alterations in intraocular pressure due to changes in resistance to outflow and those due to changes in vascular volume or in the rate of aqueous production.

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† Fellow of the Jones Optical Company, Cleveland, Ohio. Work carried out in partial fulfillment of the requirements of the Project Teaching Program of the Medical Curriculum, Western Reserve University School of Medicine.

‡ Present address: Department of Physiology, Southwestern Medical School, Dallas, Texas.

In a previous report we have described a method for removing the cat eye with a short section of its autonomic nerves intact for the purpose of recording ciliary muscle movements in the absence of vascular and extraocular muscle influences.[11] This isolated preparation furnishes an ideal system for investigation of the effects of nerve stimulation on the resistance to aqueous drainage, because, in addition to freedom from blood volume changes or extraocular muscle movements on stimulation, the uncertainties of variation in aqueous production and in capillary permeability are eliminated.

A modification of the perfusion methods of Bárány[12] and Grant[13] was used. Pressure was kept constant, and the inflow resulting from this pressure was measured. Under these conditions, provided eye volume remains constant, outflow through the filtration angle is equal to inflow to the eye.

In our experiments both the anterior chamber and the vitreous compartment were cannulated. This enabled us to raise the eye to experimental pressure by way of the vitreous compartment when this was desired. In addition, the presence of infusion solution in the vitreous compartment enabled us to measure volume changes in this compartment as a result of stimulation.

Methods

Young cats weighing 2.2 to 2.8 kg. were used in these experiments. Sodium pentobarbital, administered interperitoneally, was used as anesthesia. The freshly enucleated eye was mounted on a wax block and cannulated, as shown in Figure 1. Twenty-gauge hypodermic needle shafts were used as cannulas; one was inserted into the vitreous compartment midway between the equator and the posterior pole; the other was inserted in an oblique course into the anterior chamber just anterior to the limbus. The cannulas were conected by way of thick-walled plastic tubing to separate 200 cu. mm. horizontal pipettes which served as reservoirs. A specially designed flowmeter could

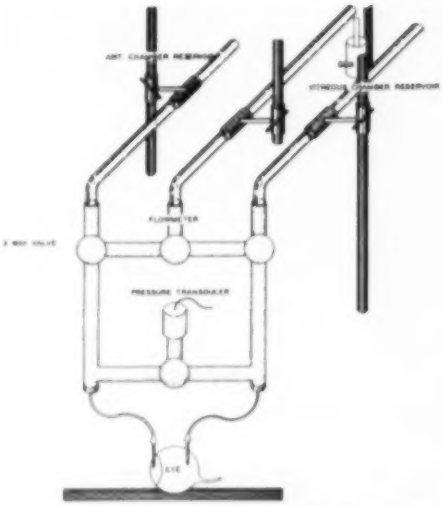

Fig. 1 (Purnell, Melton, and Adams). Connections between eye chambers, reservoirs, and flowmeter.

be substituted for either reservoir by the use of three-way valves. A Hathaway pressure transducer was so arranged that pressure could be monitored in either eye chamber as desired without cross-connecting the two cannula lines or the two reservoirs. The entire system was filled with a Kreb's solution.[14] In some experiments, fluorescein was added to the infusion solution on the vitreous side of the recording system. A filling reservoir and a calibrating manometer were additional parts of the system not shown in Figure 1. During the experiments, the eye-nerve preparation was moistened by a continuous drip of infusion solution kept at 37°C.

While the effects of stimulation on flow to or from the eye can be observed by watching the meniscus movement in a graduated pipette, the changes during stimulation are too sudden and too unpredictable to obtain an adequate record visually. To get a photographic record, a flowmeter was devised in which movement of the meniscus was translated into changes of resistance, and recorded as the deflections of a d'Arsonval galvanometer.

Details of the flowmeter are shown in

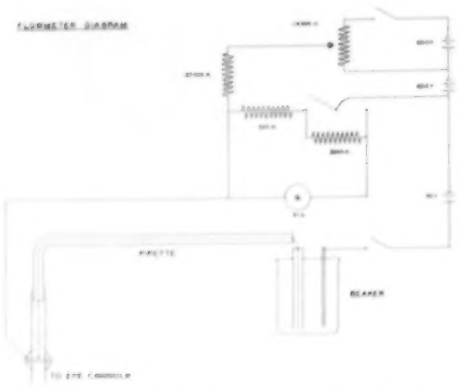

Fig. 2 (Purnell, Melton, and Adams). Details of the flowmeter.

Figure 2. A moistened silk thread passing through the lumen of a 200 cu. mm. pipette served as a variable resistance in one arm of a modified Wheatstone bridge. As fluid left the pipette to enter the eye, an additional length of wet thread was introduced into the circuit.* Electrical contact was made at one end of the pipette through the metal three-way valve; at the other end by means of an electrode immersed into a beaker of infusion solution into which the thread passed. The thread also passed through a short section of pipette within the beaker. When this section was set in proper relationship to the open end of the flowmeter, changes in electrical resistance due to evaporation from the exposed portion of thread were compensated for by the wicklike tendency of the thread to draw fluid into the pipette. Fluid level in the beaker was empirically adjusted to give a constant reading of the galvanometer. To minimize evaporation, the open ends of the flowmeter and the exposed thread were protected from room air currents by a moist chamber.

* An Ayrton shunt, comprising resistances of 850 and 8,500 ohms, and a single-pole, double-throw switch are conveniences in making preliminary adjustments or in experiments in which the total volume change is too great for recording with full sensitivity. The resistances of the various parts of the circuit are shown in the figure, but are not essential to the design.

Silver electrodes were placed on the autonomic nerve surrounding the optic nerve near the posterior pole. Stimuli were supplied by a Harvard stimulator at frequencies between 75 and 100 c.p.s., at indicated voltages between 10 and 50 volts.

Results

Pressure in the excised eye after cannulation averaged about 6.0 to 8.0 mm. Hg, depending in part upon the amount of aqueous lost in cannulation. Approximately 200 cu. mm. of infusion solution was required to raise the eyes to the pressure of 23.5 mm. Hg used in these experiments. Initial influx into the anterior chamber was instantaneous, and in three of 10 eyes brought to pressure by this route an additional inflow of 20 to 40 cu. mm. occurred when the vitreous reservoir was subsequently opened to the vitreous compartment. Initial influx into the vitreous compartment, in eyes brought to pressure by this route, took place in a matter of minutes; the inflow rate gradually slowed until it equalled the rate of anterior chamber outflow. Between 200 and 400 cu. mm. of infusion solution entered the vitreous compartment before dye was seen in the anterior chamber.

The apparent rate of outflow varied widely among the eyes used, and in the majority of cases outflow rate increased with time after the onset of perfusion. The average initial outflow through the anterior chamber in 17 eyes was 10.4 cu. mm. per minute (range 4.9 to 17.0). The maximum rate of outflow, usually obtained at the end of one hour, was 17.1 cu. mm. per minute (range 5.0 to 38.5).*

Figures 3, 4, and 5 show typical records of the effect of autonomic nerve stimulation

* In 10 experiments the eye was brought to experimental pressure by way of the anterior chamber, in seven cases by way of the vitreous compartment. The average net increase in flow rate through the anterior chamber in one hour was 13.6 cu. mm. per minute in the former (anterior) group, and 0.5 cu. mm. per minute in the latter (vitreous) group. A larger series of experiments will be needed to establish the significance of this difference.

Fig. 3 (Purnell, Melton, and Adams). Effects of electrical stimulation on inflow. Flow to anterior chamber; vitreous chamber reservoir cut out during recording. Upper tracings: signal markers and stimulus; middle tracing: pressure; lower tracing: cumulative flow.

on inflow. The lower tracing in these photographs represents cumulative flow; its slope is flow rate. The middle tracing represents pressure, the upper tracings stimulus and signal markers. Figure 3 is a record of flow into the anterior chamber. At the onset of stimulation there is a backflow of infusion fluid through the cannula and flowmeter, accompanied by a small but definite rise in pressure. Extrapolation of the prestimulus flow curve to the poststimulatory curve shows that no net change in inflow to the eye has resulted from the stimulation. Qualitatively similar results were observed in seven of 13 eyes tested.

Figure 4-A represents a second common

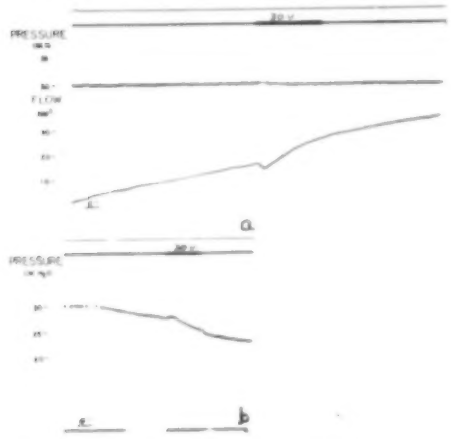

Fig. 4 (Purnell, Melton, and Adams). Tracings as in Figure 3. Record from anterior chamber. (a) Flow to anterior chamber. Vitreous reservoir cut out during recording. (b) Same. All inflow to eye was stopped at arrow.

type of response as seen in another eye. In this case there is an initial backflow of 5.0 cu. mm. during the first three seconds of stimulation, followed by an increased inflow during the remaining 19 seconds of the stimulus. After the end of stimulation flow continues, but at the original rate. An extrapolation of the prestimulatory slope falls under the poststimulatory flow curve, indicating a net increase in inflow took place during stimulation. Figure 4-B is a record from the same eye, under the same conditions of stimulation, but with reservoirs and flowmeter cut out (at the arrow). Pressure falls as fluid drains from the anterior chamber. During stimulation, however, pressure is first elevated, then falls at a faster rate until the stimulus is withdrawn. Qualitatively similar curves were obtained in six of 13 eyes tested.

Figures 5-A and 5-B record anterior chamber pressure, and flow into the vitreous compartment. In Figure 5-A the experimental pressure had been built up by way of the anterior chamber, which remained connected to the reservoir. With both chambers connected, there is a possibility of simultaneous inflow into one and backflow from the other. Inflow to the vitreous compartment during stimulation has been observed when there was no recordable pressure difference between the two chambers. The effect can be produced repeatedly if the lens-iris barrier is first driven posteriorly by manipulation of the reservoir heights. Inflow to the vitreous compartment is accompanied by a backward displacement of fluid from the anterior chamber to its reservoir.

In Figure 5-B the experimental pressure had been built up by way of the vitreous compartment, and the anterior chamber remained cut off. This was the usual type of record when flow was measured into the vitreous compartment with no input into the anterior chamber.

Pupillary reactions to stimulation were minimal or absent in these experiments; when occurring, they were only fleeting, ill-

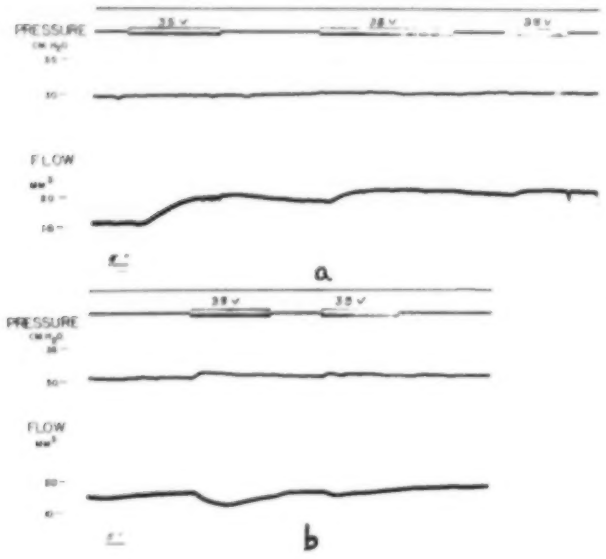

Fig. 5 (Purnell, Melton, and Adams). Tracings as in Figure 3. Record of inflow to vitreous compartment, pressure recordings from anterior chamber. (a) Anterior chamber reservoir connected to eye. (b) Anterior chamber reservoir cut out.

defined movements of the iris at the beginning of the stimulus.

Discussion

The variations in the rate of outflow among the eyes used in these experiments and the increase in outflow with time are not fully understood. The increases in outflow rate are in qualitative agreement with the published results of Bárány,[15] who noted a decrease in resistance to outflow when eyes were perfused in vitro.

The commonly employed procedure of raising the enucleated eye to experimental pressure by way of the anterior chamber should be seriously questioned. When an eye is enucleated, pressure falls immediately primarily due to loss of blood from vessels of the choroid. When fluid is added by way of the anterior chamber to bring the eye up to the desired experimental pressure, the iris is forced backward into the posterior chamber and along the contour of the lens, thus forming an effective barrier to the transfer of fluid into the retrolental space and increasing the volume of the anterior chamber.

In addition there may be displacement of the lens-iris barrier posteriorly, the magnitude of this displacement depending upon the volume of blood lost from vessels behind the lens and the resistance to lens displacement afforded by the zonule and its muscular supports. The introduction of fluid into the vitreous compartment should prevent backward displacement of the lens-iris barrier and concomitant derangement of attached structures. But even by this method physiologic relationships cannot be guaranteed, for within limits of the hyaloid membrane strength, the lens and supporting structures may be displaced in the forward direction to occupy space created by uncompensated aqueous outflow or losses entailed in anterior chamber cannulation.

A complete appreciation of the effects of autonomic nerve activity on aqueous outflow is not yet possible. However, evaluation of the data thus far collected indicates that stimulation of autonomic nerves causes pressure and volume changes in vitro. These changes cannot be explained on the basis of vascular engorgement or pupillary reactions. Since the ciliary muscle is the only other structure in the excised eye which could cause phasic changes in volume (and hence

pressure), it is concluded that activation of this structure is responsible for the results obtained in these experiments. It is possible that similar responses have been a confusing factor in experiments involving stimulation of autonomic pathways in vivo by other workers.

The changes in inflow rate of the type recorded in Figure 4-A present several interesting possibilities. Since the inflow rate subsequently returns to its prestimulus value there is no evidence for continuing effects of the stimulation, and it remains to distinguish between an irreversible alteration in the volume of the eye and a temporary change in resistance to outflow through the filtration angle during stimulation. It might be argued that the initial increase in pressure forces additional fluid through the filtration angle, and is thus responsible for the net increase in inflow seen in this record. Even if we assume the pressure elevation (approximately 1.0 cm of water) exists at its maximum value for its full duration, the net increase in volume loss through the anterior chamber angle due to increased intraocular pressure is under 0.03 cu. mm. ($C = 0.6$). The actual net increase in inflow during stimulation is 6.0 cu. mm., as determined by the upward displacement of poststimulatory slope over the prestimulatory trend line. Postulated mechanisms by which facility for outflow might be increased have been mentioned. Neither of these theories can be eliminated on the basis of present experiments.

As yet it is not possible to explain why some eyes show a net increase in inflow during stimulation whereas others do not. There is no apparent correlation of this response with the duration of the experiment, the prestimulatory rate of outflow, the manner in which the eye was raised to experimental pressure, or the experimental displacement of the lens-iris barrier. The possibility of different responses to parasympathetic and sympathetic activity cannot be excluded. Directionally opposite ciliary muscle movements have been obtained in the excised cat eye when the sympathetic and parasympathetic nerve fibers to the intraocular musculature were stimulated.[11] The difficulty in differentiating these nerve fibers without benefit of direct measurements of the muscle contraction was fully discussed in that paper. Experiments presently in progress are designed to clarify this problem through the use of autonomic blocking agents and direct muscle recording.

The apparent increase in size of the vitreous compartment shown in Figure 5-A is to be given further study. However, since this response has been observed only in eyes in which the lens-iris barrier had been experimentally displaced posteriorly, it may reflect a tendency of the lens to assume a more physiologic forward position, assisted perhaps by an alteration in zonular tension. Increased tension on the zonule due to radial muscle contraction would presumably have this effect.

Summary

1. A flowmeter capable of measuring phasic variations in flow through the perfused enucleated eye has been described.

2. Preliminary studies have been presented which demonstrate pressure and volume changes due to autonomic stimulation in the absence of extraocular influences. It is concluded that these are due to contractions of the ciliary muscle.

3. In six of 13 eyes tested there was an increase in flow into the eye during electrical stimulation.

4. An apparent increase in the size of the vitreous compartment as a result of electrical stimulation has been observed in eyes brought to experimental pressure by way of the anterior chamber and in eyes in which the lens-iris barrier was first displaced posteriorly. This may be accounted for on the basis of increased zonular tension as a result of ciliary muscle contraction.

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Discussion

Dr. Bernard Becker (Saint Louis): With improvements in and the development of better methods of measuring both aqueous flow and resistance to outflow, there has been increased and renewed interest in the reflex mechanisms that are available for the control of intraocular pressure.

Several investigators have arrived by separate pathways at the conclusion that there must be very active compensatory reflexes and interrelationships of both secretion and facility of outflow to one another. Thus, Friedenwald pointed out last year that following Diamox administration there is a decrease in the secretion which is compensated partially by a decrease in facility of outflow. From work in his laboratory by Stone and Prijot, as well as work done by Rosen, it has been demonstrated that some of the contralateral ocular reflexes inherent in tonography can be prevented by ganglionic blocking agents.

The present investigators have approached this problem with a new point of view. It is to be emphasized that they are using the enucleated cat's eye to study the effects of nerve stimulation. As Dr. Purnell has demonstrated in his presentation, their instrumentation is ingenious. Working under these in vitro conditions, the changes induced in ocular volume and facility of outflow can be studied without the influence of blood supply or aqueous secretion. By stimulating nerve endings in such enucleated cat eyes, they have been able to induce small rises in intraocular pressure—rises of comparable magnitude to that induced by Dr. von Sallmann in stimulating the diencephalon.

The interesting question must be raised as to whether or not such a method of raising intraocular pressure would explain in part some instances of the rise in intraocular pressure Dr. von Sallmann reported this morning. You must remember (again, to emphasize) that here we are dealing with an enucleated eye, where alterations in blood volume and alterations in secretory activity have been eliminated.

I think the other point that might be made in this regard is the finding of Dr. von Sallmann, as reported this morning, that there was no change in radiophosphorus uptake or radiophosphorus content of the eye following diencephalic stimulation to raise intraocular pressure. He concluded that the radiophosphorus method of Bettman and coworkers does not measure blood flow. The question must be raised, however (and this is only a question at present which needs further exploration), as to whether the rise in intraocular pressure, induced by stimulating the diencephalon, is always truly a vascular response, or whether it might in part be related to the rise in pressure that Dr. Purnell has just reported.

It has really been a privilege to read this paper ahead of time and to listen to it today. I am fascinated by their provocative, preliminary work on a most challenging question. It is hoped that this important approach will be further explored so as to amplify our understanding of the regulatory mechanisms that control intraocular pressure.

SPECIES VARIATION IN FACILITY OF AQUEOUS OUTFLOW*

BERNARD BECKER, M.D., AND MARGUERITE A. CONSTANT, PH.D.
Saint Louis, Missouri

The perfusion of enucleated eyes permits the evaluation of the facility of outflow (C) of aqueous from the anterior chamber of the eye.[1,2] This method can be applied to eyes regardless of size, shape, corneal curvature, or scleral rigidity. When modified for use in the living animal, estimates of intraocular pressure (P_o) can also be obtained.[3] The in vivo data for rabbit and human eyes agree well with the in vitro results as well as with the findings of tonography for the same eyes. A comparison of the perfusion values for the very different sized eyes of cats, guinea pigs, and rabbits revealed striking variations in outflow facility. The differences observed suggested a relationship between facility of outflow and volume of the anterior chamber. The results of studies on outflow facility and its variation with anterior chamber volume and species differences are presented in this report.

METHOD

Rabbits weighing two to three kg., cats weighing three to four kg., and guinea pigs weighing 600 to 700 gm. were anesthetized with Nembutal or urethane and heparinized. Cannulation and perfusion were carried out in the living animal as well as in the freshly enucleated eyes as previously described.[3] Human eyes were obtained at autopsy and were perfused eight to 144 hours after death. At each infusion pressure (P_I), the rate of buffered saline† inflow (I) was measured until a constant value was obtained. Testicular hyaluronidase (Wydase®) 15 TRU/ml. or bacterial hyaluronidase (Hyason®)‡ 15 U/ml. were used in some infusions.

The in vivo data were analyzed graphically by plotting I, the rate of inflow in cu. mm./min. against P_I, the inflow pressure in mm. Hg. Straight lines were obtained with slope equal to C, the facility of outflow, and which intercepted the pressure axis (zero inflow) at the initial intraocular pressure, (P_o). This method of interpretation was based upon the equation:

$$I = C\ (P_I - P_o) \qquad (1)$$

where

I = inflow rate (cu. mm./min.) corrected to 37°C.
P_I = inflow pressure (mm. Hg)
P_o = intraocular pressure in the undisturbed eye
C = facility of outflow

In the enucleated eye the equation simplified to:

$$I = C \times P_I$$

RESULTS

The data obtained from the in situ eyes of living cats indicated an average intraocular pressure of 22 mm. Hg and facility of outflow of 1.54. The in vitro data for enucleated cat eyes revealed a facility averaging 1.62 and differed from the in vivo values on the same eyes by an average of only 10 percent (table 1). Similar results for guinea pigs are summarized in Table 2. Here the intraocular pressure averaged 20 mm. Hg with a facility of outflow of 0.08 in vivo and 0.09 in the enucleated eye. In the 18 experiments where data were available

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† Isotonic sodium phosphate and potassium dihydrogen phosphate, pH 7.4, diluted 1:10 with isotonic saline.

‡ The Hyason used in this study was kindly supplied by Dr. Kenneth W. Thompson of Organon, Inc., Orange, New Jersey.

TABLE 1

COMPARISON OF IN VIVO AND IN VITRO ESTIMATES OF FACILITY OF OUTFLOW IN CATS

	In Vivo		In Vitro	
	P_o	C	C	ΔC
	25	1.20	1.14	0.06
	27	1.55	1.80	0.25
	20	1.12	1.27	0.15
	23	1.25	—	—
	25	1.20	1.40	0.20
	20	1.15	1.25	0.10
	24	1.10	1.40	0.30
	24	2.00	—	—
	23	1.70	1.68	0.02
	22	1.45	—	—
	20	1.30	—	—
	25	0.91	0.95	0.04
	25	1.40	1.44	0.04
	20	2.20	—	—
	19	2.10	2.35	0.25
	18	1.88	1.94	0.06
	15	2.25	2.30	0.05
	15	2.00	2.10	0.10
Mean	22	1.54	1.62	0.13
No. Eyes	18	18	13	13

ΔC = absolute difference between in vivo and in vitro values for C
P_o = intraocular pressure (mm.Hg)
C = facility of outflow

TABLE 2

COMPARISON OF IN VIVO AND IN VITRO ESTIMATES OF FACILITY OF OUTFLOW IN GUINEA PIGS

	In Vivo		In Vitro	
	P_o	C	C	ΔC
	19	0.05	—	—
	15	0.17	0.15	0.02
	15	0.11	—	—
	21	0.10	0.11	0.01
	18	0.11	—	—
	20	0.10	0.13	0.03
	20	0.07	—	—
	25	0.08	0.10	0.02
	15	0.07	—	—
	17	0.08	—	—
	24	0.08	0.09	0.01
	26	0.05	0.05	0.0
	17	0.07	—	—
	—	—	0.08	—
	—	—	0.08	—
	24	0.09	0.12	0.03
	15	0.14	—	—
	23	0.06	0.06	0.0
	25	0.05	0.06	0.01
	22	0.05	—	—
	19	0.07	—	—
	19	0.07	0.08	0.01
	20	0.04	0.07	0.03
	19	0.08	—	—
	20	0.09	0.09	0.0
	20	0.06	—	—
	18	0.09	0.10	0.01
	22	0.08	0.08	0.0
	17	0.06	0.08	0.02
	20	0.05	0.08	0.03
	22	0.10	0.09	0.01
	20	0.06	—	—
	17	0.10	0.11	0.01
	19	0.06	—	—
	17	0.05	—	—
	20	0.09	—	—
Mean	20	0.08	0.09	0.014
No. Eyes	34	34	20	18

ΔC = absolute difference between in vivo and in vitro values for C
P_o = intraocular pressure (mm. Hg)
C = facility of outflow

on the same eyes, the in vivo and in vitro values for facility differed by only some 15 percent. Data obtained previously[3] for rabbits revealed an average pressure of 19 mm. Hg with facility of 0.35. Representative eyes of each species in vivo are presented graphically in Figure 1. The mean data for all three species are summarized in Table 3. It was apparent at once that although the eyes of all three species had similar average intraocular pressures, there were enormous (almost 20-fold) differences in facility of outflow.

This comparison could be carried one step further by computing the species differences in rate of aqueous secretion.* Mean values for cats, rabbits, guinea pigs, and humans are summarized in Table 4. It is evident that F, the rate of secretion of aqueous, varied enormously in these species from an average of only 0.8 cu. mm./min. for the guinea pig to 18.5 cu. mm./min. in the cat eye. However, when flow was expressed as per-

* Flow (F) in cu. mm./min. was calculated from the equation:

$$F = C\ (P_o - P_v) \qquad (2)$$

where P_v is the episcleral venous pressure; P_o is the intraocular pressure; and C = facility of outflow. Assuming a mean value of 10 mm. Hg for P_v, flow rates were determined from the data in Table 3 or estimated graphically from the inflow vs. pressure plots (the flow value at a pressure of 10 mm. Hg). Average tonographic values for intraocular pressure and facility of outflow in a series of 175 normal human eyes were included for comparison.

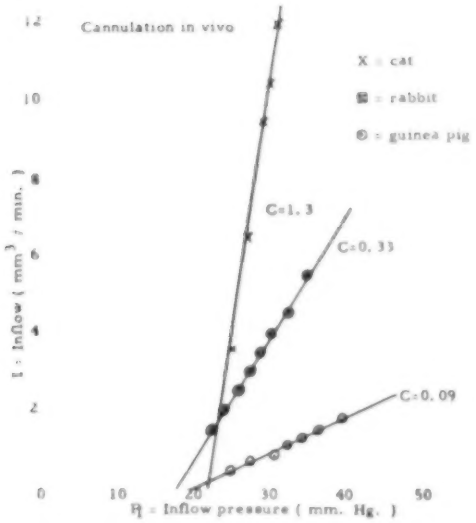

Fig. 1 (Becker and Constant). Graphic representation of species variation in facility of outflow (C) and intraocular pressure.

cent of the volume of the anterior chamber per minute (K_f), it became apparent that all four species had very similar values for the flow constant K_f (1.3 to 1.6 percent).

That the values for K_f were similar for the eyes of different species was noted by Bárány.[4] Using the para-amino hippuric acid method for measuring the flow constant K_f, he reported values of 1.4 to 1.7 percent for guinea pigs, 1.4 percent for cats, and 1.1 to 1.3 percent for American rabbits. Furthermore, Goldmann[5] found a value of 1.3 percent for human eyes from turnover studies with fluorescein. The agreement of estimates of aqueous flow obtained by such different

TABLE 3

PERFUSION MEASUREMENTS—SPECIES VARIATION

Animal	No Eyes	P_0	C_{vivo}	C_{vitro}
Guinea pig	34	20	0.08	0.09
Rabbit	53	19	0.35	0.37
Cat	18	22	1.54	1.62

P_0 = intraocular pressure (mm. Hg).
C_{vivo} = facility of outflow in vivo.
C_{vitro} = facility of outflow in enucleated eye.

TABLE 4

SPECIES VARIATION

	P_0	C	F	V	K_f
Guinea pig	20	0.08	0.8	0.050	1.6%
Rabbit	19	0.35	3.2	0.200	1.6%
Cat	22	1.54	18.5	1.200	1.5%
Man	17[a]	0.31[a]	2.2	0.175[b]	1.3%

P_0 = intraocular pressure (mm. Hg).
C = facility of outflow.
F = aqueous flow (cu.mm./min.), assuming episcleral venous pressure 10 mm. Hg.
V = volume anterior chamber (ml.) (estimated from volume obtained from anterior chamber puncture).

$K_f = \frac{F}{10\ V}$ = flow constant (percent of anterior chamber per minute)

a = Mean tonographic values for 175 normal human eyes.
b = Value for volume of anterior chamber of human eyes is average of Goldmann's measurements.[5]

approaches as turnover studies, tonography, and cannulation perfusion, each with its own errors and assumptions, is supportive evidence for the validity of the methods of measurement.

The similarity of average values for intraocular pressure and the coefficient of aqueous flow, K_f, for different species indicated a direct correlation of facility of outflow with anterior chamber volume.* From Table 4 it is apparent that in the four species considered in spite of the large differences in facility of outflow (C) and anterior chamber volume (V), the average values for C/V all fell between 1.3 and 1.8.

These findings raised entertaining speculations as to the stability of the parameter K_f in an individual eye. The question of the

* If the volume of the anterior chamber (V) is expressed in ml., from equation (2) it is apparent that:

$$K_f = \frac{F}{10\ V} = \frac{(P_0 - P_v)\ C}{10\ V} \qquad (3)$$

Thus, if K_f were to remain constant in a given eye without producing alterations in intraocular pressure (P_0) or episcleral venous pressure (P_v), the facility of outflow (C) must vary directly with the volume of the anterior chamber (V) or

$$\frac{C}{V} = \text{constant} \qquad (4)$$

constancy of C/V for any one eye was, therefore, subjected to experimental investigation. This relationship could be approached most readily by perfusion of the enucleated eye. Under these conditions, the volume of the anterior chamber could be varied at will by moving the iris lens diaphragm forward or backward. This was accomplished by the introduction of a measured amount of saline into the vitreous cavity as described by Purnell[6] or by a clamp about the sclera posteriorly. The alteration in anterior chamber volume was measured readily by the rapid change in the site of the meniscus in the anterior chamber pipet.

In Figure 2 are plotted the variations of facility of outflow (C) with alterations in volume of the anterior chamber in two representative enucleated human eyes. It is readily seen that reasonably straight line plots were obtained for each eye with slopes (C/V) equal to 1.2 and 1.5 respectively. In Table 5 the estimates obtained by such facility vs. volume plots for 32 enucleated human eyes are summarized. Values for C/V varied from 0.9 to 2.5 in this series of eyes, averaging 1.5. This was in reasonable agreement with the in vivo value of 1.8

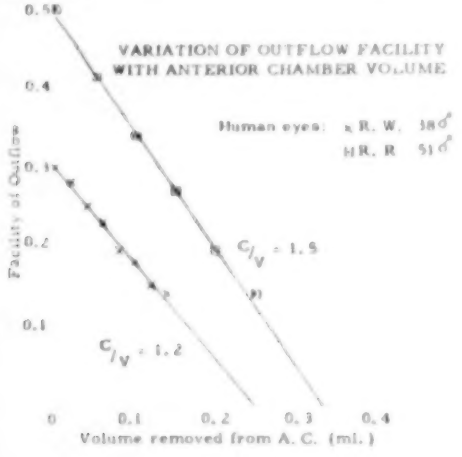

Fig. 2 (Becker and Constant). Variation of facility of outflow with alterations in volume of anterior chamber in two representative enucleated eyes.

TABLE 5

THE RATIO* C/V IN 32 ENUCLEATED HUMAN EYES

Patient	Age & Sex	Time (hr.)†	C/V
O.D.	89 M	9	1.0
A.P.	45 M	44	1.3
		46	1.2
W.P.	49 M	50	1.1
		52	1.2
R.W.	38 M	25	1.2
		28	1.3
J.R.	51 F	43	1.9
E.S.	44 M	70	1.4
S.S.	64 F	5	1.6
		8	1.8
G.W.	42 F	22	1.4
		70	1.4
J.D.	63 M	20	1.7
		72	1.6
A.M.	79 F	20	1.8
		20	1.9
J.F.	87 M	44	1.2
		44	1.4
W.H.	39 F	66	1.3
K.P.	60 F	8	1.1
		144	1.1
V.H.	58 M	70	2.1
		70	2.0
E.H.	68 F	76	1.7
W.C.	70 M	40	2.2
		40	2.5
V.H.	58 F	40	1.8
		40	2.0
K.P.	7 F	140	1.0
		140	0.9
R.R.	51 M	30	1.5
Average (32 eyes)			1.5

* Ratio C/V determined from slope of straight line plot of variation of facility of outflow (C) with volume of anterior chamber in ml. (V) in vitro.

† Approximate time after death.

calculated from average tonography and estimates of mean anterior chamber volume of normal eyes.[5] Alterations in outflow resistance with variations of depth of the anterior chamber in enucleated human eyes have been reported recently by François.[7] Although the data of these workers cannot be quantitated as to variation of resistance with changes in volume of the anterior chamber, they do appear to agree qualitatively with the above findings.

Similar studies of the variation with anterior chamber volume of the outflow facility in the perfused rabbit eye were carried out. The same linear relationship of C and V was readily demonstrated. Bárány and Woodin[8] had reported alterations in out-

flow facility following perfusion with hyaluronidase. The question arose as to the interrelationships of the hyaluronidase and volume induced alterations in facility of outflow. In Figure 3 are presented a selected facility vs. volume plot in a rabbit eye perfused with buffered saline without and then with the addition of hyaluronidase 15 TRU/ml. It is apparent that linear relations of C and V were obtained before and after hyaluronidase. Of particular importance was the finding that the hyaluronidase effect on facility was not the result of change in anterior chamber volume, but a true increase in the ratio C/V. Data recorded in Table 6 revealed an average value for C/V of 1.7 for 17 enucleated rabbit eyes and 2.4 for eight of these after hyaluronidase. Thus, at least two independent mechanisms for alteration in outflow facility appear to be available in *enucleated rabbit eyes*—the reversible change with anterior chamber volume and the permanent effects of hyaluronidase.

These findings on enucleated eyes are of considerable theoretical interest for they suggest the possibility that in the normal living eye alterations in anterior chamber depth or volume may also be associated with changes in outflow facility. The role of this potential factor in some of the induced and spontaneous changes in facility observed in vivo deserves exploration. If these facility modifications occurred at constant levels of intraocular pressure and in similar fashion to those in enucleated eyes, this would offer a mechanism for maintaining a relatively constant coefficient of flow, K_f. Thus, the rate of aqueous secretion expressed in percent of the volume of the anterior chamber may well remain reasonably constant not only among different species, but for the same eye under different conditions. This would provide a unique method for maintaining a uniform concentration rather than total amount of a constituent of the aqueous humor. The clinical significance of such speculation remains obscure, however.

Tonographic data do not indicate any gross correlations in patient populations of facility of outflow and volume of the anterior cham-

TABLE 6

THE RATIO C/V IN 17 ENUCLEATED RABBIT EYES AND EFFECT OF HYALURONIDASE

Before Hyaluronidase C/V	After Hyaluronidase C/V
0.8	1.5
0.9	—
1.8	2.2
1.5	—
1.6	2.2
1.2	—
1.0	2.1
1.0	—
1.8	3.0
2.1	—
1.7	2.6
2.2	—
2.4	2.4
2.6	—
2.0	3.1
2.2	—
2.5	—
Average 1.7	2.4
No. Eyes 17	8

C/V = slope of plot of outflow facility (C) vs. anterior chamber volume in ml. (V).

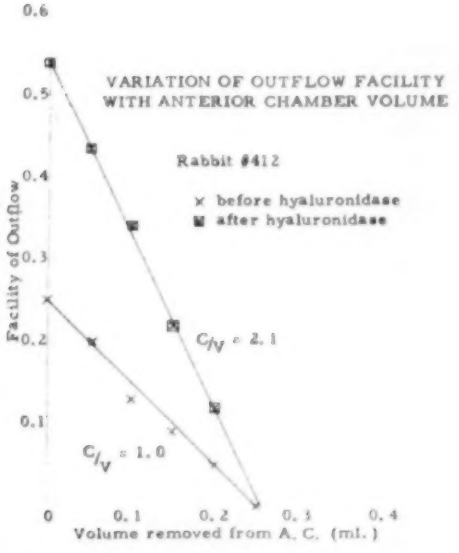

Fig. 3 (Becker and Constant). The effects of hyaluronidase upon the variation of outflow facility (C) with anterior chamber volume (V) in a single rabbit eye.

ber. The large anterior chamber of aphakic eyes as well as the very small volume of anterior chamber in some "narrow angle" eyes do not appear to be associated with significant alterations in outflow facility unless synechiae are present. However, François[9] has recently noted in one human eye increasing values of C associated with deepening of the anterior chamber in vivo. It is clear that more data are needed as to the relationship of facility of outflow and volume of the anterior chamber in the living eye. In the enucleated eye, the mechanism whereby outflow facility is altered with changes in anterior chamber volume should prove a fruitful field for investigation.

Summary

1. Perfusion measurements of facility of outflow (C) in vivo reveal average values for the cat of 1.54, the rabbit 0.35, and the guinea pig 0.08 in spite of very similar values for intraocular pressure in all three species.

2. In vivo and in vitro values for C on the same eyes differ by only some 10 to 15 percent.

3. Although absolute rates of aqueous flow vary greatly in the eyes of the guinea pig (0.8 cu. mm./min.), the rabbit (3.2 cu. mm./min.), man (2.2 cu. mm./min.), and the cat (18.5 cu. mm./min.), the rate of secretion of aqueous expressed as percent of the volume of the anterior chamber is remarkably constant at 1.3 to 1.6 percent for all four species.

4. Facility of outflow varies in linear fashion with the volume of the anterior chamber not only among different species, but even more precisely in the individual enucleated human or animal eye.

640 South Kingshighway (10).

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Discussion

Dr. Elmer J. Ballintine (Cleveland): The authors sent me a copy of this paper over a month before this meeting so that we had plenty of time to study it and actually repeat some of the experiments.

The authors are modest for the experiments are more difficult than they appear to be from the description in the text. However, we were able to confirm, in general, the results.

The authors emphasize the similarity of "C" when measured by perfusion in vivo and after enucleation. It should also be emphasized that while the "C's" are similar, the values measured in vitro are consistently higher than in vivo by about seven to 12 percent, depending on the species. The reason for this discrepancy requires further investigation.

In our experiments on freshly enucleated eyes, the linear relationship between "C" and volume of the anterior chamber were studied only after the perfusion had been continued for about one-half hour. In the first half-hour the value of "C" was erratic, and lower than the values obtained subsequently. The reasons for this initial low "C" are not apparent.

ELABORATION OF BICARBONATE ION IN INTRAOCULAR FLUIDS*

III. Vitreous humor following intravenous infusion of 1.3 percent $NaHCO_3$

H. Green, Ph.D., M. J. Mann, A.B., and J. L. Sawyer
Philadelphia, Pennsylvania

In the second paper[1] of this series it was shown that the bicarbonate ion concentration in the vitreous humor adjacent to the ciliary body was higher than that in the rest of the vitreous humor (31.3 mM/L compared to 24.0 M/L) and that the concentration in the peripheral ring was equal to that in the central core. On the basis of such static distribution definite conclusions with regard to the route of entry of the bicarbonate ion into the vitreous humor could not be drawn.

In connection with experiments designed to study the effect of physiologic variations of blood bicarbonate levels upon the concentration in the intraocular fluids, it was found that under carefully controlled conditions the continuous intravenous infusion of isotonic $NaHCO_3$ solution raised the arterial blood plasma level from a normal average of about 20 mM/L to about 30 mM/L in 60 minutes, which level remained steady for at least four hours.[2] At the same time the bicarbonate ion concentration in the aqueous humor of the anterior chamber rose to 35 mM/L and that of the posterior chamber to 44 mM/L and they remained steady for at least four hours. Such conditions of elevated levels of bicarbonate ion concentration in the aqueous humors and in the blood plasma, it was thought, afforded us the opportunity of experimentally evaluating the factors responsible for the distribution and the route of entry of the bicarbonate ion in the vitreous humor of the rabbit eye.

* From the Department of Research, Wills Eye Hospital, and the Department of Physiology Chemistry, Graduate School of Medicine, University of Pennsylvania. This investigation was supported in part by a Grant-in-Aid from the National Council to Combat Blindness.

Experimental procedure

Adult pigmented rabbits weighing between four and six pounds were used. The continuous intravenous infusion of 1.3 percent $NaHCO_3$ was conducted by means of the usual venoclysis assembly as previously described.[2] Immediately after withdrawal of the aqueous humor and of the blood from the ear artery* the animal was killed by air embolism. Both eyes were then enucleated, frozen, and sectioned, and bicarbonate ion concentration determined as previously described. All analyses were run in duplicate. A diagrammatic sketch of the sections of vitreous humor is shown in Figure 1. In this study the vitreous humor in the area of the ciliary body was compared with the rest of the fluid.

Results

It is clear from the results in Table 1 that the concentrations of bicarbonate ion in the two sections of the vitreous humor are steady during the one- to five hour period. This corresponds to the steady-state conditions present in the blood plasma and aqueous humor of the anterior and posterior chambers under the identical experimental conditions as previously reported.[2] A comparison with the average values[1] of the normal distribution of bicarbonate ion presented in Table 2 reveals that while the concentration in ciliary body area of the vitreous humor remained essentially unchanged, that in the rest of the vitreous humor showed a slight but statistically significant increase. This is further borne out by the fact that the normal

* Results of the analyses of these fluids comprise part of the data in the first paper of this series[2] referred to in the introduction.

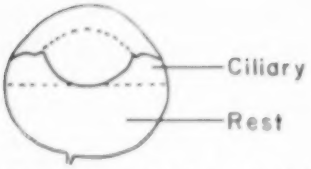

Fig. 1 (Green, Mann, and Sawyer). A diagrammatic sketch of the sections of the vitreous humor.

ratio of the concentration in the ciliary body area to that in the rest of the vitreous humor changed from an average of 1.34 to 1.2.

Comments

On the basis of measurements of the concentration of thiocyanate and radioactive sodium ions in the aqueous humor of the posterior chamber at different periods of time following intraperitoneal injection, Kinsey, Palm, and Cavanaugh[3] suggested "that at least one fast and one slow process were operative in controlling the filling of the posterior chamber." In excess of 30 to 40 minutes the slower process became dominant. The physiologic equivalent of the slower process was believed to be the "slow filling of the 'environments' (the vitreous, lens, and other tissues posterior to the ciliary body)." Failure to take into account this diffusional exchange with the environment would lead to serious errors in the methematical treatment of the dynamics of the aqueous humor of the posterior chamber.

If the above formulation is correct, then an increase in the concentration of an ionic species in the aqueous humor of the posterior chamber should lead to an increase in the concentration in the posterior environment. Assuming that the vitreous humor represents an important segment of the environment in diffusional exchange with the posterior chamber fluid, one should reasonably expect the increased concentration in the posterior chamber fluid to result in an increased concentration in the vitreous humor, especially in that part of the vitreous humor which is immediately adjacent and posterior to the ciliary body and posterior chamber.

TABLE 2

Normal distribution of bicarbonate ion concentration in vitreous humor of rabbit eye

	Ciliary mM/L	Rest mM/L	Ciliary / Rest
Average	31.3	24.0	1.34
St. Deviation	3.5	2.6	0.25
No. of Samples	18	18	18

TABLE 1

Bicarbonate concentration in rabbit vitreous humor after continuous intravenous infusion of 1.3 percent $NaHCO_3$

One Hour			Two Hours			Five Hours		
Ciliary mM/L	Ciliary Rest mM/L	Ciliary / Rest	Ciliary mM/L	Ciliary Rest mM/L	Ciliary / Rest	Ciliary mM/L	Ciliary Rest mM/L	Ciliary / Rest
29.6	28.9	1.02	31.7	22.9	1.38	33.5	25.5	1.31
36.1	29.8	1.21	31.1	30.2	1.03	28.8	28.5	1.01
22.5	29.9	0.75	27.8	27.6	1.01	32.4	22.0	1.47
34.4	28.4	1.21	34.3	25.0	1.37	27.2	23.1	1.18
33.9	29.5	1.15	30.6	22.5	1.36	30.3	29.4	1.03
32.6	27.3	1.19	32.1	23.9	1.34	29.4	26.7	1.10
30.8	27.6	1.12	34.0	33.2	1.02	33.6	28.3	1.19
34.4	24.9	1.38	36.9	32.9	1.12	27.5	26.3	1.05
24.0	31.8	0.75	29.3	27.6	1.06	30.9	26.9	1.15
31.7	25.2	1.26	32.2	24.8	1.30	32.0	30.5	1.05
36.2	16.2	2.23	27.9	—	—	35.6	28.0	1.27
31.4	27.4	1.15	27.5	21.2	1.30	24.3	26.4	0.92
						32.4	30.2	1.07
						27.2	23.6	1.15
						32.4	24.8	1.31
						29.6	23.1	1.28
						30.2	27.0	1.12
Average 31.5	27.2	1.20	31.3	26.5	1.21	30.4	26.5	1.16
St. Deviation 3.5	3.8	0.11	2.6	3.9	0.14	2.9	2.4	0.13

The results reported herein indicate that the bicarbonate-ion concentration of the vitreous humor immediately adjacent to the ciliary body and posterior chamber was not affected by an increase in concetration of about 18 percent (from 36.5 to 43 millimoles per liter) in the posterior chamber and by an increase of about 50 percent (from 20.0 to 30 millimoles/liter) in the arterial blood plasma.

Since the elevated concentration in both blood plasma and aqueous humors was obtained within one hour and remained steady for the next four hours, it is hardly likely that the experimental time period was too short to permit a significant amount of diffusional exchange. If, however, such an objection could be raised, it is apparent that the rate of diffusion from the posterior chamber aqueous humor to the vitreous humor is too slow to have any appreciable effect upon the dynamics of the aqueous humor. It, therefore, seems reasonable to conclude that it is improbable that any significant net exchange occurs between the bicarbonate ion in the aqueous humor of the posterior chamber and that in the vitreous humor of the rabbit eye. The data do not permit any conclusion to be drawn with regard to diffusional exchange of the bicarbonate in the lens either with the aqueous humor or with the vitreous humor.

Concerning the rest of the vitreous humor the results show that the bicarbonate ion concentration increased from 24.0 to an average of 26.7 mM/liter within one hour and the level remained steady for four hours. This increase of 2.7 millimoles/liter (11.2 percent) is statistically significant. It is assumed that this increase in bicarbonate ion concentration arose chiefly by diffusion from the choroidal and retinal circulation, because of the increase in blood plasma bicarbonate from 20mM/liter to 30 mM/liter. Comparative analyses of the sections of the vitreous humor under these conditions would, therefore, probably show that the increase in bicarbonate ion concentration occurred chiefly in the peripheral ring.

Two fundamentally important questions remain unanswered:

1. The source of the excess bicarbonate ion concentration in the vitreous humor between the ciliary body and the lens compared with that in the rest of the vitreous humor.

2. The source of the excess present in the latter section of the vitreous humor compared to that in the blood plasma.

Concerning the first question, for reasons discussed in paper II and in the light of the present results, it appears unlikely that the bicarbonate ion diffuses into the vitreous humor from the posterior chamber, at least to any appreciable extent. Although the results do not permit any conclusion to be drawn concerning diffusional exchange of bicarbonate ion in the intraocular fluids with the lens, anatomic and biochemical considerations suggest that this organ may play a significant role in maintaining the optimal ionic milieu of the aqueous humor and also participate in ionic transfer with the vitreous.

Summary

1. Isotonic sodium bicarbonate (1.3 percent) was continuously infused intravenously into adult pigmented rabbits weighing between four and six pounds, over a period of five hours.

2. The bicarbonate ion concentration of the arterial blood plasma rose from an initial level of 20 mM/L to 30 mM/L and remained steady for four hours.

3. The bicarbonate ion concentration in the aqueous humor rose from 30 to 35 mM/L in the anterior chamber and from 36 to 43 mM/L in the posterior chamber during the first hour and remained steady for the next four hours.

4. The bicarbonate ion concentration in the vitreous humor lying between the lens and the ciliary body was unchanged, while that in the rest of the vitreous humor increased significantly 11.2 percent.

5. It is suggested that no significant net exchange occurs between the bicarbonate ion in the aqueous humor of the posterior chamber and that in the vitreous humor of the rabbit eye.

6. The data do not permit any conclusion to be drawn with regard to diffusional exchange with the lens.

1601 Spring Garden Street (30).

ACKNOWLEDGMENT

We wish to express our appreciation to Dr. Irving H. Leopold, Director of Research, for his encouragement and helpful discussion during the course of this investigation.

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DISCUSSION

DR. JOHN E. HARRIS (Portland, Oregon): This nice piece of work has so many facets that I find it rather hard to open the discussion. I have chosen, therefore, to touch very lightly on a few of the aspects, and emphasize that which I think is perhaps the main aim of their studies, namely, to shed further light on the mechanism by which bicarbonate gets to the posterior chamber.

Considering, first of all, the vitreous values, Dr. Green is aware, I know, that others have found the bicarbonate concentration of the whole vitreous body to be lower than plasma. The present authors' normal plasma values are somewhat low. I would, however, tend to agree with their conclusions. One would in general anticipate that the vitreous body would have a higher concentration of bicarbonate than arterial plasma.

The question of whether there is exchange between the posterior chamber and the adjacent vitreous body, I think, has been answered in the affirmative for sodium, by Kinsey and his associates and by other workers. The results of Green and associates certainly would indicate that such an exchange of bicarbonate is, at best, slow. The authors killed their animals by air embolus. These animals die a violent death, and it is feasible that a slight difference might be nullified by such a procedure.

The question of the dynamics of the anterior chamber deserves some comment, although I anticipate Dr. Kinsey will have further word to say on that. (Slide) We are considering here just the dynamics in the anterior chamber, and we are not considering at all the kinetics of exchange in the posterior chamber. It is well to emphasize that these equations, and all such equations, are tools and not rules. As Friedenwald and Becker aptly stated, they represent man's best physiologic intuition. To them we must apply the data. If reliable data do not conform, then we must get ourselves a new equation. This has been done several times through the years.

I would wish to point out here that this equation has been found by both Kinsey and Palm and by Friedenwald and Becker to fairly well describe the movement of sodium and thiocyanate into and out of the anterior chamber, both groups using the data gathered by Kinsey and Palm. It does not appear to describe the movement of bicarbonate as here measured. It does not of necessity follow that the basic concept embodied in the equation is erroneous. There are certain other factors not expressed which always have to be considered and can only be ignored in such simplified equations when adequate proof permits. In this instance I would like to mention two factors. The first is the question of another reservoir, not included in the equation, e.g., the cornea. It is possible that exchange between this reservoir and the anterior chamber will vary with the ion. Second, there are certain physiologic functions which must remain constant during the experimental situation. Among these are the vascular mass, the rate of blood flow and surface area across which diffusion occurs. Alteration of these functions will cause K_B to vary. Such alteration may occur when bicarbonate is injected. Under such circumstances the CO_2 tension would rise and we have seen today from the work of Dr. Bettman and his associates that when the CO_2 tension is raised, the vascular mass in the eye increases. Thus K_B is not of necessity constant even though Drs. Green and Leopold found no change in the protein in the anterior chamber.

To me the most important question is: How does the bicarbonate enter the eye, or more specifically, the posterior chamber? The authors have found that the rise in bicarbonate in the posterior chamber was not proportionately as great as that in the plasma. The kinetic equations that can be employed to define the movement of a substance that is found in higher concentrations in the aqueous than the plasma are at best inexact. However, it would be informative to consider two concepts, the first of which assumes that the movement of the constituent is proportional to its plasma concentration and the second which assumes that the secretory activity is independent of the plasma level.

The first concept is employed in the expression of Kinsey and Palm, who considered that the kinetics of a secretory mechanism were such that the entrance into the posterior chamber was proportional to the plasma concentration of the actively transported constituent. If one applies their equation to the data of Green and his associates one finds that the concentration of bicarbonate in the posterior chamber should have risen by an amount of approximately 20 milliequivalents per liter. Instead, it rose only in the neighborhood of 8 milliequivalents per liter. One can conclude, therefore, that the data

do not fit the equation, that the active movement of bicarbonate into the posterior chamber is not proportional to the plasma concentration.

The other major possibility I would like to consider is that the accumulation of bicarbonate is independent of the plasma concentration. Friedenwald's original concept, as a matter of fact, considered that such activity was actually a vegetative process of the ciliary body. This vegetative mechanism can be likened to the basal metabolic rate. It would conceivably put a constant amount, N, of bicarbonate into the posterior chamber per unit time. This rate would be independent of the plasma level. In addition to the active transport one must consider a possible diffusional exchange between the posterior chamber and the plasma and the balance that is achieved because of flow, the flow resulting from the osmotic differential established when N molecules of dry substance are deposited in the posterior chamber. In terms of amounts then, our equation becomes:

$$\frac{dA_{p.c.}}{dT} = N + k\,(C_{pl} - C_{p.c.}) + V_{fl}\,(C_{pl} - C_{p.c.}).$$

Dividing now by the volume of the posterior chamber the equation becomes:

$$\frac{dC_{p.c.}}{dT} = N + k_d\,(C_{pl} - C_{p.c.}) + K_{fl}\,(C_{pl} - C_{p.c.}).$$

Fundamentally the equation postulates a constant and active introduction of bicarbonate into the posterior chamber with a superimposed gain from plasma proportional to the plasma concentration and loss from the posterior chamber proportional to the concentration in the posterior chamber. Applying the authors' data, at the new steady-state the concentration in the posterior chamber would be expected to increase 11 milliequivalents per liter. The measured increase was 8.0 milliequivalents per liter.

The equation as listed has certain inadequacies which are not pertinent to our major thesis and correction for which is not warranted by present information. First, the exchange with the environment has been ignored. Second, the factor of flow of bicarbonate into the posterior chamber is taken to be proportional to the plasma concentration. A more correct expression would be k_{fl} (n C_{pl}) where n is a fraction which may be one or less. A value of less than one would lower the predicted concentration. Lastly, the effect of rising CO_2 tension on the diffusion exchange may be of some significance; we do not know.

In all, the data best fit the concept of a constant production of bicarbonate, independent of plasma concentration with a superimposed exchange with plasma. The work therefore supports the theories of Dr. Friedenwald. Whether we believe that carbonic anhydrase is required for the active transport is essentially immaterial; it is a detail. The essential feature of his concept is an organized system of oxidation-reduction enzymes spatially oriented at molecular distances which bring about the accumulation of a substance into the posterior chamber. We may eventually find that actually bicarbonate is only secondarily transferred—that there may be a cation that is primarily secreted. That will in no way dim the luster of his brilliant concept.

Dr. Harry Green: The development of our understanding of the dynamics of aqueous humor formation has been attendant upon the introduction of new experimental data and explanatory theories and speculation. As new facts become available the basis for a more adequate understanding is provided. Similarly, when the prevailing theory or assumptions are no longer compatible with the experimental data, then a revision of our thinking must be made. All will agree, I am sure, that the ultimate validity of any theory rests upon experimental verification. Toward that end considerably more experimental work must be done before the secrets of the complexities of the dynamics of the intraocular fluids are revealed.

SPECTRO-DIFFERENTIAL ELECTRORETINOGRAPHY IN MACULAR DISEASE*

Jerry Hart Jacobson, M.D., Demir Basar, M.D., and Abraham L. Kornzweig, M.D.
New York

The fact that the retina produces an electrical potential change in response to photic stimulation which can be detected and recorded has been known for many years. In the past 10 years the measurement of this potential in humans has become a relatively simple matter, thanks to the development of a suitable electrode. The clinical applications of the physiologic phenomenon have gradually been elucidated and are becoming well known.

Most of the work in the past on clinical

* From the Department of Electrophysiology, New York Eye and Ear Infirmary. This study was supported by grants from the Arnold Reuben Fight for Sight Fund of the National Council to Combat Blindness, and the National Society for the Prevention of Blindness. Some of the patients were obtained as part of a study, in conjunction with Dr. A. L. Kornzweig, of the eye in the aged, under Department of Health, Education and Welfare Grant number B 154.

applications has been in the field of retinal degeneration, such as retinitis pigmentosa, siderosis retinae, and, with numerous reservations, retinal detachment. In each of these disease entities there exists a generalized retinal dysfunction, and significant alterations of the electroretinogram were found in each of them.

Another field of interest, however, has been in the attempt to evaluate, by use of the electroretinogram, the functional ability of the retina when it is obscured by medial opacity, such as cataract, corneal opacity, or vitreous opacity. In these situations, where ophthalmoscopic view of the fundus is usually unsatisfactory or impossible, some means of determining retinal status by means other than the patients' subjective reports would be of great value.

Using the techniques of Karpe,[1] who is the father of modern clinical electroretinography, we were able to obtain a record in these cases. The record obtained, however, is a measure only of total retinal, and, hence, predominantly rod function. It has been shown by numerous investigators that the electroretinogram as recorded under the conditions standardized by Karpe was a measure of the scotopic function only, and could not be relied upon to give a measure of macular function.

It was apparent to those of us who have been working with this technique that some means of differentiation of the photopic from the scotopic function would greatly enhance the practical benefits to be derived from clinical electroretinography. The approaches to the problem of this separation of rod from cone have been as follows:

The first attempt, by several authors, including Karpe himself, was to restrict the area of the stimulating light to a pencil of light which would affect only a given area. The conclusion generally drawn from this work was that there is scatter of the impinging light, either by the ocular media, or the contact lens, or perhaps by lateral neuronal connections in the retina, to the extent that the stimulus could not be readily localized.

The other approaches utilize known facts about the differences in physiologic behavior of the photopic and scotopic mechanism. The difference in chromatic sensitivity of the two, the photopic mechanism being relatively more sensitive to the long end of the spectrum, and the scotopic low in reaction to this red stimulus, has been known for many years. This is the basis of the work to be reported. It has been learned, in recent years, that there is a difference in the flicker-fusion frequency between photopic and scotopic mechanism and also, clinically, between peripheral and central retina. It has been shown that the fusion frequency of the scotopic mechanism is about 25 per second and that of the photopic mechanism is up to 70 per second.

Accordingly, attempts are being made to utilize this differential to "uncover" the photopic reaction. This technique will use an intermittent stimulus of a frequency higher than that of scotopic fusion, but below that of photopic fusion frequency. By the use of a frequency analyzer, which amounts to a filter which allows electrical impulses of only one definite frequency to pass, we will be able to find the reactions which are occurring each time the stimulus is applied, and filter out the remaining interference. This work is being done at our laboratory and also by Dr. Henkes of Holland, in conjunction with Dr. Riggs of Brown University.

It has also been known for many years that the scotopic response increases greatly under dark adaptation, and is diminished by light adaptation. Attempts have been made to utilize this fact by adapting the eye to a strong background illumination and then using an even stronger light as a stimulus.

Finally, combinations of the phenomena are possible, such as preadaptation with a strong monochromatic light and then using another monochromatic light of flickering character as stimulus.

The modification of the test to include these new techniques has required modification of the equipment, as well.

The original technique and equipment of

Karpe were quite simple. The patient was dark adapted for a period of five or 10 minutes, and then a stimulus light, which was a 25-watt bulb, was flashed by a relay for one-seventh second. This incandescent type of bulb requires time to heat up and begin to glow, so that the duration of the actual stimulus was about one twenty-fifth second. We use the word "about" deliberately, since, to quote Karpe,[2] "the intensity produced by incandescent bulbs, even of the same theoretical wattage, is very variable and it is necessary to calibrate each bulb." Karpe used a modified portable electrocardiograph machine, and an incandescent light source, similar to a desk lamp, controlled by a relay. The test was performed in ordinary clinical surroundings.

In our newer apparatus we have found it necessary to enclose the patient in a shielded, grounded chamber. This allows us to eliminate the ambient electrical interference due to power lines, and X-ray and diathermy equipment which have been found to be a major source of interference in the measurement of the minute quantities of electricity now being investigated (fig. 1).

The stimulus we now use is a stroboscope furnishing 3.2 million candlepower. This much illumination is needed to obtain sufficient luminous flux after it is passed through a deep filter. The stroboscopic light source also allows us to stimulate at frequencies up to 100 per second, with complete ease and accuracy of adjustment. We now use a cathode ray oscilloscope to monitor the tracings. Because of the complete lack of inertia in this recording device, there is none of the distortion of high frequency responses inherent in any electro-mechanical device such as a galvanometer or ink writer. A timing device co-ordinates all the stimulating and recording sections. This is shown schematically in Figure 2.

The stimulus light is approximately 20 inches from the patient, and provision is made for the interposition of filters between the patient and light source. The stimulating light is so situated and shielded that it is impossible for any light except that from the stroboscope to impinge upon the eye being tested.

The potential is picked up by a silver electrode in a contact lens, and led to the

Fig. 1. (Jacobson, Basar and Kornzweig). Patient in shielded chamber, recording instruments in background.

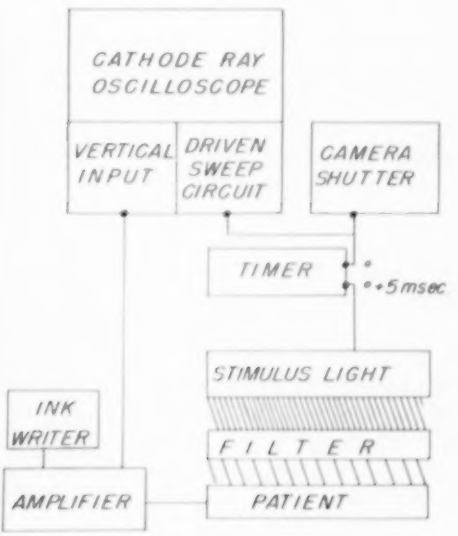

Fig. 2. (Jacobson, Basar and Kornzweig). Scheme of stimulating and recording circuits.

input of the Grass electroencephalogram machine X-amplifier. One channel of the electroencephalogram is connected to a cathode ray oscilloscope input, utilizing the electroencephalogram as a pre-amplifier. The remaining channels of the electroencephalogram are utilized to drive the direct writing pens. The "EMG" setting, utilizing the shortest possible time constant available, is used in all instances.

The work with color differential electroretinogram dates back to 1949, when Riggs and Johnson[3] of Brown University showed that there was a difference in response to red, green, and blue stimuli. In the red stimuli it was apparent that there was a short, biphasic response not elicited with the other colors. Donner[4] also showed that in isolated retinal elements, with a microelectrode technique, red stimuli produce an earlier frequency maximum of spike discharges than other colors.

Motokawa and Mita, in 1942,[5] and Schubert and Bornschein,[6] and Armington[7] have demonstrated, under varying conditions, the existence of an x-wave, really the first, fast, probably cone response of the b-wave of Granit and Karpe. This was present in night-blind individuals but not in protanopes. Cobb and Morton in 1952[8] and Auerbach[9] in 1954 have shown that there is probably a duality to both the a- and b-waves, with a fast component of each corresponding to photopic function, and a slower one due to scotopic mechanism activity.

Accordingly, with this knowledge of a color differential existing in the responses of the electroretinogram, and with the knowledge that cones were more sensitive to the deep red end of the spectrum than the rods, it was decided to test the reaction of patients with macular disease of such a nature as to lead us to suspect that a proportion, at least, of the cone population of the retina, were not functioning normally, and to compare them to normal individuals.

As preliminary, reactions to several filters were tried. The reactions to Wratten filters 75 and 74 were essentially the same as those to white light of equal intensity. Of the red filters, Wratten number 29 and number 70 were studied, and a commercial gelatin filter. The last was finally used, since it was easily available in large enough size. It has a cut off point of significant transmission at 610 millimicrons.

About 50 normal eyes were tested with this stimulus, of which 14 are here presented as a representative sample. The patients were each allowed 10 minutes of dark adaptation and then stimulated with white and colored stimuli. These are tabulated as in Figure 3.

In each of these normal eyes, as well as those eyes with cataract but otherwise normal, a definite response to red light was obtained. This was of a lower order of magnitude than that due to white light, but always definitely present and measurable.

In several cases the potential was so small that we were concerned with the possibility that we were measuring a photoelectric response of the oxide of the electrode metal. Careful analysis has ruled this possibility out.

There is a wide age span in our normal group. The responses to the red light varied from 10 to 45 microvolts, and were almost always identical in each eye. We next obtained tracings in a series of 37 patients with various types of macular lesions, in which it is generally assumed that the loss of vision is due to disruption of the photopic function. We have simply called these senile macular degenerations. By this is meant any one of the several types of degeneration in older individuals which are visible ophthalmoscopically and are, in the opinion of the examining ophthalmologist, the cause for reduction of vision to 20/200 or less. These are tabulated in Figure 4.

None of these patients had any measurable response to red light, although their responses to white light were of normal or only slightly reduced magnitude. Case 106 is particularly interesting. This is a man with a unilateral macular hole. The involved eye

ERG NO	AGE	SEX	DISEASE	VISUAL ACUITY OD	VISUAL ACUITY OS	ERG (MICRO VOLTS) WHITE L OD	ERG (MICRO VOLTS) WHITE L OS	RED L OD	RED L OS
56-011	39	F	CATARACT O D	CF	20/40	225	200	30	30
55-178	40	F	CATARACT O D	HM	20/50	200	200	20	20
56-003	60	M	CATARACT O D	20/400	20/40	170	185	15	15
55-037	57	F	CATARACT O S	20/50	CF	225	225	25	25
55-180A	44	M	CATARACT O D	5/200	20/20	175	175	20	20
55-123	46	M	NORMAL	20/20	20/20	225	275	30	30
55-103	28	M	NORMAL	20/30	20/30	225	225	20	20
55-105	32	M	NORMAL	20/30	20/30	175	200	15	20
55-120	60	M	NORMAL	20/20	20/20	150	150	15	15
55-084	80	M	NORMAL	20/30	20/30	150	150	15	15
55-085	56	M	NORMAL	20/30	20/40	150	150	20	20
55-181	70	F	NORMAL	20/30	20/30	225	225	15	15
55-162	21	F	NORMAL	20/30	20/20	250	250	40	40
55-192	48	M	NORMAL	20/20	20/20	210	210	30	30

Fig. 3 (Jacobson, Basar and Kornzweig). Reactions of normal retinas to white and red light stimuli.

ERG NO	AGE	SEX	DISEASE	VISUAL ACUITY OD	VISUAL ACUITY OS	ERG (MICRO VOLTS) WHITE L OD	ERG (MICRO VOLTS) WHITE L OS	RED L OD	RED L OS
55-122	67	M	SENILE MAC DEG	20/50	20/50	100	125	EXT	EXT
55-119	50	F	MAC DEG	20/50	20/50	150	125	EXT	EXT
55-163	42	F	MAC DEG	20/70	20/70	150	125	EXT	EXT
55-106	63	M	MACULAR HOLE,OS	20/20	20/200	175	125	15	EXT
55-200	70	M	SENILE MAC DEG	20/100	20/100	175	175	EXT	EXT
55-133	58	M	MAC DEG	20/30	20/200	125	125	20	EXT
55-130	59	M	SENILE MAC DEG	20/200	20/200	100	100	EXT	EXT
55-127	81	M	KUHNT JUNIUS, OD	20/200	20/70	15	25	EXT	EXT
55-151	79	M	SENILE MAC DEG	CF	20/200	25	150	EXT	EXT
55-155	74	M	KUHNT JUNIUS, OD	5/200	CF	175	175	EXT	EXT
55-128	88	F	SENILE MAC DEG	10/200	2/200	35	35	EXT	EXT
55-129	70	M	SENILE MAC DEG	HM	5/200	25	110	EXT	EXT
55-164	87	M	SENILE MAC DEG	20/200	20/200	50	75	EXT	EXT
55-165	80	F	SENILE MAC DEG	20/200	10/200	75	110	EXT	EXT
55-166	77	F	SENILE MAC DEG	20/400	20/70	75	100	EXT	EXT
55-170	73	F	SENILE MAC DEG	20/200	20/200	100	100	EXT	EXT
55-175	80	F	SENILE MAC DEG	20/200	20/200	75	200	EXT	EXT
55-180	78	M	SENILE MAC DEG	20/200	20/200	220	100	EXT	EXT
55-188	85	M	SENILE MAC DEG	20/200	20/200	165	150	EXT	EXT
55-199	86	M	SENILE MAC DEG	20/200	20/200	200	200	EXT	EXT
55-201	60	M	SENILE MAC DEG	10/200	10/200	130	150	EXT	EXT
55-198	80	F	SENILE MAC DEG	20/200	20/200	125	125	EXT	EXT

Fig. 4 (Jacobson, Basar and Kornzweig). Reactions of retinas with macular disease to white and red light stimuli.

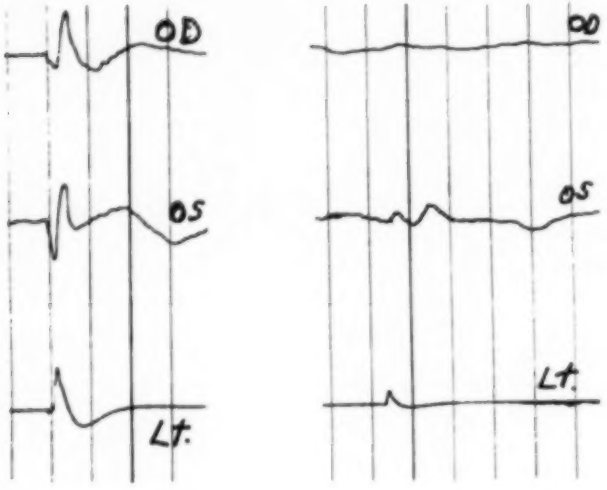

Fig. 5. (Jacobson, Basar and Kornzweig). Reactions of patient with macular hole of right eye to white (left) and red (right) stimuli.

shows no response to red light, but the other is normal. This unilaterality of reaction is also seen in Case 133, where the right eye is normal and has a normal response to red light but the left eye, with macular degeneration, is extinguished.

The average latent period in response to red light was 9.1 milliseconds, the time to achieve maximum b-wave was 17.2 milliseconds, and the average duration was 46 milliseconds.

In several instances, the findings illustrated in Figure 5 were found. Where a unilateral macular disease was present—in this instance a macular hole in the right eye, and a normal left eye—a larger a-wave was present in the normal eye.

Summary and Conclusions

It has been found that in those eyes with severe macular disease, reducing the central acuity to 20/200 or less, there is an absence of response to a deep red stimulus.

Although we cannot assume that these cases of macular disease had a complete destruction of their photopic mechanism, we can assume that, due to altered internal environment, a large part of this mechanism is reduced in efficacy. Certainly not all the cones in a patient with macular degeneration are destroyed, in fact most of these patients in our series were able to recognize the color of the stimuli. However, the change in circulation, or hormonal balance, or whatever is basically the cause of the macular disease may so affect the individual units responsible for the photopic mechanism throughout the eye that the electrical activity is perceptibly altered.

It is our feeling that this alteration may be detected in this manner, or in combination with other techniques enumerated above, so as to provide a reliable means of judging macular function in an eye whose fundus cannot be seen.

800 Fifth Avenue (21).

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Discussion

Dr. Werner K. Noell (Buffalo, New York): Dr. Jacobson's paper has aptly demonstrated the usefulness of electroretinography in retinal disease. I am in no position to evaluate the applicability of electroretinography in clinical ophthalmology, but I am tempted to compare clinical electroretinography with clinical electroencephalography, which is my daily business since many years.

As I see it, the main impact of clinical electroencephalography on neurology was by providing a better understanding of the pathogenesis and symptomatology of certain cerebral diseases. The vigorous and devoted application of electroencephalography to clinical problems led to a better evaluation of the patient's symptoms and to better diagnosis of, for instance, the various types of epilepsy and this mainly for the reasons that electroencephalography provided a wealth of new information on the patient's disease in general and that physician and electroencephalographer acquired this new knowledge in close contact with each other and with the experimental discipline.

In a similar manner we should consider and apply electroretinography and we should expect that its greatest benefit will derive from its application as a clinical research tool.

There are, of course, pitfalls in this business of electroretinography. Many of these are due to the fact that the electroretinogram is a response and that the stimulus parameters influence the response. I agree with Dr. Jacobson that his findings indicate the diffuse impairment of the photopic mechanism in these macular diseases. However, I think the question of whether or not this impairment is co-existent with the full preservation of scotopic mechanisms will need further studies designed specifically for testing scotopic responsiveness with stimuli of low intensities.

I was particularly interested in the last slide which showed (and one could see it also in the others) that the a-wave was reduced in response to a strong flash of white light. We observe a similar phenomenon in rabbits under conditions which either lead to or are associated with a degeneration of the outer limbs.

We quantitate this phenomenon by the b/a ratio. Plain loss of visual cells reduce a- and b-waves but does not affect the b/a ratio. On the other hand, retinal pathology which preferentially involves the outer limbs is associated with a selective depression of the a-wave. The b/a ratio is then increased and the b-wave may even be higher in amplitude than in a comparable control. In animals in which the b/a ratio has been high in vivo, we also find often histologic signs of pigment epithelial damage but this does not hold true for all experiments nor does it hold true for all experimental procedures which produce a high b/a ratio.

If pigment cell damage is present we are inclined to assume that the outer limbs became affected secondarily; but obviously both possibilities—primary versus secondary involvement—exist in every case. It was comforting to note that Dr. Jacobson's thinking fits so well with our own efforts in analyzing experimental results and that his data actually contained a phenomenon which somehow links together clinical and experimental data. I want to congratulate him for this valuable piece of work.

EFFECT OF HALLUCINOGENIC DRUGS ON THE ELECTRORETINOGRAM*

JULIA T. APTER, M.D.
Chicago, Illinois
AND
CARL C. PFEIFFER, M.D.
Atlanta, Georgia

For the past eight years psychiatrists have been interested in certain hallucinogenic drugs. Since these drugs induce visual hallucinations and changes in the personality in normal human subjects, they have been called "psychotomimetic."

Mescaline is one such drug. Its effects have been well described in the published literature.[5] Its property as psychotomimetic was not emphasized at first. Lysergic-acid diethylamine tartrate (LSD-25), however, is another such drug and has been thought to induce a state resembling schizophrenia.[4, 10, 11, 12]

Up till now, neurophysiologists have studied the effects of these drugs on the brain exclusively.[2, 7-9] A perusal of the clinical facts, however, indicates that the retina may be involved in the production of the drug-induced visual hallucinations:

Firstly, patients with optic-nerve blindness do not experience the visual hallucinations.[1, 3]

Secondly, the character of the hallucinations is best explained on the basis of intraocular structures.

The characteristics common to all drug-induced visual hallucinations are lattices, honeycombs, and blue-red arcs.[5, 6, 11]

Thirdly, Kluver[6] has shown that this type of visual experience may be produced by rubbing the eyeball and by exposing the eye to flickering or to intensely bright light.

Because of the associated systemic changes, however, these three facts have not suggested to neurophysiologists that the retina should be studied. They have heretofore confined their efforts to studies of the brains of experimental animals.

It should be stressed that these drugs could not be designated as psychotomimetic unless the brain was the site of origin of the visual hallucinations. It is essential, therefore, that the extent to which the retina participates in this drug action be determined. The results obtained from this study directed toward that end is of great interest to ophthalmologists also. This presentation will describe the unique effects the drugs have on the electrical activity of the visual system of cats.

The study is based on the principle that an electrical potential accompanies activity in any nervous element. Action potentials may be detected anywhere on the surface of the eyeball following marked changes in the illumination of the retina.[4] These potentials indicate activity in the retina and indicate that retinal elements are transmitting impulses. It follows, therefore, that such a retinogram would be induced by the hallucinogens when the illumination of the eye remained constant only if the retina were participating in the production of the spontaneous visual experiences. It seemed worthwhile, therefore, to investigate the effects of these drugs on the retinograms of cats.

METHOD

I. Forty-four cats were anesthetized with intraperitoneal pentobarbital (42 mg./kg.). Two nonpolarizable silver-silver chloride electrodes were sutured under the conjunctiva of each cat and served to pick up potentials from the retina through the sclera. A Grass electroencephalograph recorded these potentials. The efficacy of this procedure in demonstrating the activity of the retina

* From the Manteno State Hospital, Manteno, Illinois, and the Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia.

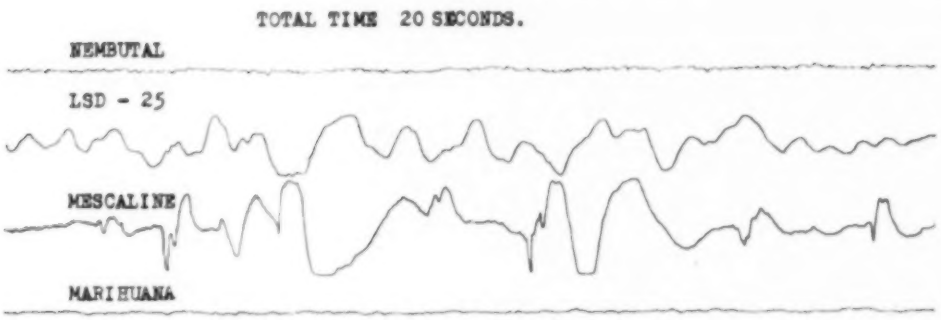

Fig. 1 (Apter and Pfeiffer). Spontaneous potentials from each of four cats, each under the influence of a different drug. (Up is positive.)

was tested by noting the potentials recorded following photic stimulation of the retina. Since evoked potentials appeared consistently at the onset and cessation of this stimulation, this method of pick-up and recording was deemed adequate for this study.

The effects of LSD-25 (25μ/kg.), mescaline (50 mg./kg.), pentobarbital (40 mg./kg.), and dihydrocannabinol (2.0 cc. per cat) on the retinogram and on the resting potentials of the eye were compared in an effort to determine whether the hallucinogenic drugs act directly on the retina.

II. In nine cats, two electrodes were wound around the optic nerve of one eye. In these cases all muscle and nerve connections of that eye were severed to eliminate any contribution nonvisual elements might make to nerve action potentials. The other eye of each of these cats had electrodes applied as in Method I. LSD-25 was injected and its effect on potentials in the optic nerve was studied.

III. In seven cats, two electrodes were placed on each eye as in Method I. Simultaneously a bipolar electrode was placed for pick-up from an exposed area on the occipital visual cortex. The effect of LSD on potentials in this area was noted. Then the effect of section of the optic nerves on the induced LSD-effect was recorded.

IV. In order to ascertain a nontoxic dose of LSD for cats and to determine whether cats experience visual hallucinations with it, 0.10 mg. of LSD-25 was injected into the peritoneal cavity of each of three unanesthetized and unrestrained cats. Their behavior was observed for two hours. In no case were the cats dark adapted.

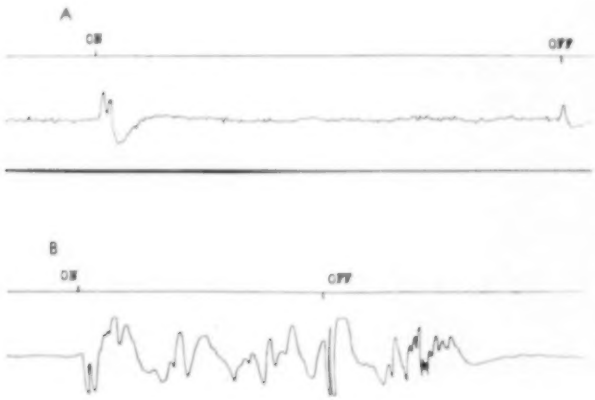

Fig. 2 (Apter and Pfeiffer). (A) Potentials induced by turning a light on and off the cat's retina while anesthetized with Nembutal. (Up is negative.) (B) 15 minutes after administration of 0.10 mg. of LSD-25. (Up is positive.)

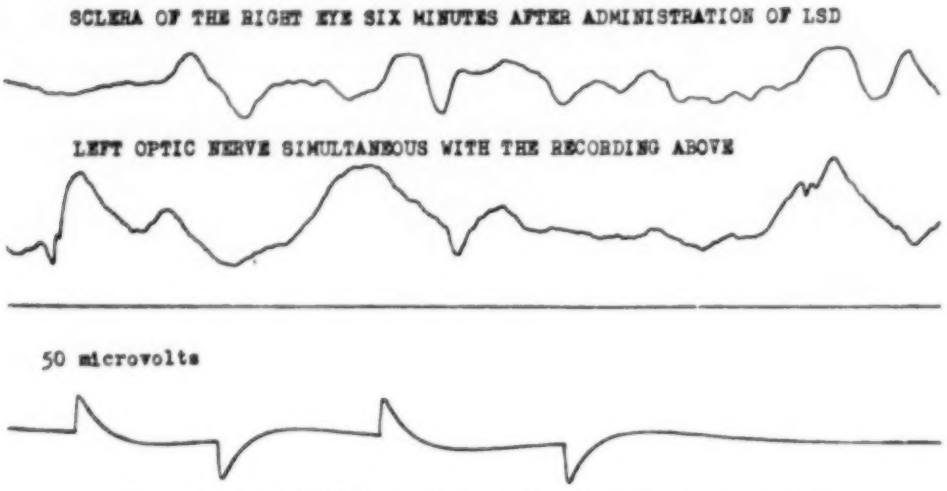
SCLERA OF THE RIGHT EYE SIX MINUTES AFTER ADMINISTRATION OF LSD
LEFT OPTIC NERVE SIMULTANEOUS WITH THE RECORDING ABOVE
50 microvolts

Fig. 3 (Apter and Pfeiffer). Simultaneous pick-up from optic nerve and retina showing spontaneous potentials. (Up is positive.)

Results

I. All 44 cats had a normal resting electroretinogram and a normal response to photic stimulation for about eight minutes. At that time striking changes occurred in the 22 cats that had received LSD or mescaline.

In these cats spontaneous potentials appeared although the illumination to the eyes remained constant. These spontaneous potentials appeared of low amplitude at first (25 microvolts) and increased in amplitude to about 200 microvolts and then gradually decreased in intensity and disappeared.

This build-up and recession of spontaneous potentials was called a "paroxysm." Each paroxysm lasted about 40 seconds and consisted of as many as 50 sharp waves of short duration. Intervening resting periods lasted 60 to 100 seconds. The first or second paroxysm lasted longer and showed higher voltage of potentials than subsequent paroxysms. A single dose of LSD induced about seven to 10 paroxysms of spontaneous potentials.

During the resting periods and for an hour after the paroxysms had ceased, photic

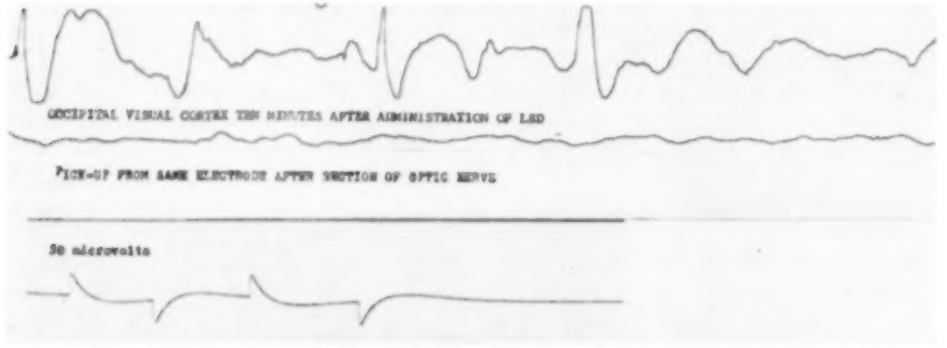
OCCIPITAL VISUAL CORTEX TEN MINUTES AFTER ADMINISTRATION OF LSD
PICK-UP FROM SAME ELECTRODE AFTER SECTION OF OPTIC NERVE
50 microvolts

Fig. 4 (Apter and Pfeiffer). Pick-up from occipital visual cortex 10 minutes after the administration of LSD-25 before and after section of the optic nerve. The top record shows spikes induced by LSD; the record below it shows the absence of spikes after section of both optic nerves. (Up is positive.)

stimulation of the retina induced a prolonged evoked electroretinogram. This was characterized by two or three waves (300 microvolts) immediately following the onset of the light and other waves (50 to 150 microvolts) continuing throughout the duration of the photic stimulation. The off-response was of higher voltage than before the drug was administered and was also prolonged.

II. Since the eyes and the animals were immobile during this activity, it did not seem likely that these were motor phenomena. To be certain, however, that these potentials were in the sensory visual pathway, the eye was severed from all muscle and nerve connections except the optic nerve. Pick-up from the optic nerve produced the same picture as the pick-up from the sclera. Section of the optic nerve stopped the potentials in the proximal stump but not in the peripheral stump of the nerve.

III. In four cats pick-up was made from the occipital visual cortex and the sclera simultaneously. Spontaneous spikes appeared on the cortex after ocular paroxysms started. After section of the optic nerves the cortical spikes ceased but the ocular spikes continued for at least four minutes.

IV. In three unanesthetized and unrestrained cats the dose of LSD was administered intraperitoneally and the behavior of the cats was observed. For six minutes the cats rubbed their eyes vigorously. This stopped after four more minutes. Their pupils and lid slits were widely dilated. While the cats rested in a normal crouching position, they moved their heads around in irregular circles as though peering at objects. They moved rapidly backward as though startled by something. They waved a front paw in front of their face as though grasping at some object. This behavior strongly suggested that the cats were having visual hallucinations and it occurred 10 minutes after intraperitoneal administration of LSD. The spontaneous scleral and optic nerve potentials appear 10 minutes after administration of the drug, also.

Conclusions

These experiments demonstrate that a nontoxic dose of LSD-25 induces changes in behavior in cats. This same dose of LSD initiates spontaneous action potentials in the retina of anesthetized cats. The potentials are certainly not induced by muscle activity. Moreover, the cortical activity induced by the drug is dependent upon nervous transmission from the retina and does not appear when the connection with the retina is severed.

These findings are consistent with the clinical data. The objective description of geometric hallucinations induced by LSD in reliable human subjects is best explained on the basis of intraocular structures. In humans with section of the optic nerve, LSD does not induce hallucinations. These facts, coupled with the experimental data, demonstrate that LSD does not induce hallucinations by virtue of an effect on the central nervous system. The facilitation on central synapses produces no effect on the visual pathways unless a visual experience starts in the retina. This facilitation which we have demonstrated in the retina and which Purpura[7] reports in the geniculate body and cortex with mescaline accounts for the complex hallucinations which sometimes appear. They, too, depend on an intact connection from the eye.

These findings lead us to conclude that LSD hallucinations are not similar to psychotic hallucinations. They also introduce a new subject. The study of the effect on the electroretinogram of various hallucinogenic drugs that do not have the undesirable side-effects of LSD and mescaline could prove fruitful in determining the site of pathology in retinal disease.

Summary

1. Spontaneous action potentials appear on the sclera, optic nerves, and occipital visual cortex of cats 10 minutes after systemic administration of lysergic acid diethylamine or mescaline, but not Nembutal or marihuana.

2. Cats seem to experience visual hallucinations 10 minutes after systemic administration of LSD.

3. The optic nerve and cortical potentials cease central to section of the optic nerve.

4. The scleral potentials do not cease after section of the optic nerve.

7316 South Paxton Avenue (49).

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Discussion

Dr. Albert M. Potts (Cleveland): That a study of visual hallucinants might be another lever to pry loose a few more facts about ocular neurophysiology from very resistant bedrock has occurred to a number of us, and Dr. Apter's paper has projected us right into the middle of that particular quarry.

The most striking effect, as far as I was concerned, was the fact that the spontaneous cortical potentials are dependent upon the integrity of the optic nerve under the conditions of these experiments. A fascinating aspect is the suggestion that the geometric nature of the hallucinations may be dependent upon the geometry of the receptor cell mosaic.

There are, however, a number of cautions which should be observed before coming to any final conclusions about the mechanism of action of these drugs. The first of these has to do with the dosage used. Dr. Apter's animals, receiving 100 μg. LSD per cat and 150 mg. of mescaline per kg. of cat, were receiving 10 or more times the customary human dose, and the mescaline dose was an LD^{100} for these animals.

So, we are dealing with a drug effect, at a dosage a whole order of magnitude higher than that used in humans. As a matter of fact, if one assumes a two to four kg. cat and a dose of something like 25 mg. per kg., the customary hallucinogenic dose for humans at 0.5 to 1.0 mg. per kg. is close to one fiftieth of the amount used in these experiments.

The features shown in the electroretinogram are also curious to us in the light of work that we have been conducting, particularly with mescaline, along the same lines. Dr. Wiedenthal and I have been working on the subject for six months now. Our mescaline dosages were approximately 15 mg. per kg., one tenth of that used in the paper reported. We find something that is considerably different.

Instead of a response that is characterized by an unusual spike in the negative deflection, followed by the spontaneous potentials, we find on our doses—which are much lower, to be sure—an apparently nonspecific accentuation of the a-wave compared to the normal and complete abolition of the b-wave.

This, as you may remember, was an effect similar to that observed on the administration of formaldehyde. We have no explanation for its basic physiology, but it seems to bear little relationship to the effect reported in the paper. This certainly is a big area for exploration.

The effect described by Rovetta should also be thought of in connection with the spontaneous potentials, particularly in the cortex, observed here. Rovetta found that local application of strychnine or mescaline to the cortex caused evocation of spontaneous potentials. We might well think of these effects as comparable to those of the photoanaleptic synergism in causing seizures. Here the dosage of analeptic drug itself (ordinarily metrazol) is not at seizure level but, when light stimuli are added, the net result is epileptiform electroencephalograph findings plus epileptic seizures.

It is quite conceivable that in the same way the dosage of hallucinogenic drugs at these high levels causes a lowering of the irradiation threshold so that the light stimulus causes another unusual

thing—spontaneous potentials in the central nervous system.

In the light of this report, and previous ones, there seems to be a considerable amount to be learned about how the visual system works from a study of hallucinants and their antagonists. However, a great deal of this study still lies before us.

Dr. Werner K. Noell (Buffalo, New York): A year or so ago when it was suggested that Reserpine might exert its action via the serotonin system, we tested in a few experiments on rabbits whether serotonin or lysergic acid would affect the electroretinogram, specifically whether these agents would interfere with the electrically measured function of the pigment epithelium. Dr. Ruedemann, Jr., and I had just found that adrenalin produces specific changes of this function but this effect was rather small with low doses and we wondered whether any other hormone might control the ion transport function of the mammalian pigment epithelium. Moreover, the peripheral circulatory effects of Reserpine are somewhat similar to those of sodium azide and sodium azide has the most powerful action upon the electrical processes which depend upon pigment epithelial functioning.

The experiments to which I referred had negative results, that is, the electroretinogram (a-, b-, and c-waves) were not specifically affected by these agents and the D.C. potential across the eye did not change appreciably nor did the response to azide. I am quite thrilled, therefore, that Dr. Apter's experiments, which differ from ours with respect to animal species and pick-up technique, were so obviously successful. We may have done something wrong. But, it would be interesting to know whether the spontaneous activity which Dr. Apter recorded was associated with an increase in the D.C. potential across the eye. If this were the case, caution should be exercised in the interpretation of A.C. fluctuations because a high D.C. potential across the eye renders the A.C. recording susceptible to all kinds of artefacts; I then also would prefer optic tract recordings to recordings from the optic nerve; changes in neuronal discharge activity I would determine by microelectrode recordings from the retinal ganglion cells.

I would like to mention a further experience of ours. Dr. Petersen and I just completed a study on the retinal effects of trichloroethylene, "Trilene." Trilene is a powerful analgetic and frequently used in neurosurgery. We were interested in its cerebral effects but to our great surprise we found—as it has happened often in our laboratory—outstanding retinal changes. I would like to mention the ability of this agent to increase the amplitudes of the electroretinogram, to transform a photopic electroretinogram into a scotopic one, and to increase the synchronization of certain retinal activities. Here, then, is another agent—known and used for its psychic effects—which affects retinal function and which permits us to analyze phenomena and mechanisms of its action on a much simpler system than the brain. I am certain that once the biologic reality of the phenomena which Dr. Apter recorded have been proven beyond any doubt, it will be possible to pinpoint their anatomic and physiologic origin. I agree with Dr. Potts, that Dr. Apter's studies deserve the most serious consideration.

Dr. Jerry Hart Jacobson (New York): After talking to Dr. Apter a few months ago about her interesting findings, I decided to try this on a few humans. We have been able to find only one cooperative individual so far, and unfortunately he did not have hallucinations; so we are in a bit of difficulty.

We did, however, give one human being 50 mg. of LSD, and recorded for a period of two hours his spontaneous reactions and his reactions to light stimuli.

It was impossible to judge any outcome from his spontaneous activity. As any of you who have done any clinical retinography know, the slightest eye motion produces a potential very similar to that which was seen here; and in so far as the reactions to light stimuli were concerned, we could find no effect in this particular individual.

Again I would like to emphasize that he did not suffer hallucinations.

Dr. Julia T. Apter (Manteno, Illinois): I want to thank the discussers. I think their contributions all were most applicable to the problem of the effect of drugs on the electroretinogram.

I do feel that even if these spontaneous potentials in the retina are due (as Dr. Noell says) just to a breakdown of an increase of a drug-induced D.C. potential that has been produced by the A.C. amplifier; even that will stimulate us to find out more about the meaning of action potentials on the retina in other cases, and in normal eyes. Perhaps we do not yet know the full significance of action potentials. This particular drug and others like it may open the field and help us to discover more about that.

However, since the spontaneous potentials induced by LSD in the electroretinogram occur only when the retina is illuminated (they are not present in the dark) this would indicate that these spontaneous potentials are indicative of spontaneous (not evoked) retinal activity. In that case we might assume that the retina participates in the production of visual hallucinations induced by LSD-25.

PHYSIOLOGIC STUDIES WITH FLICKER ELECTRORETINOGRAPHY*

GEORGE GOODMAN, M.D., AND GILBERT ISER, M.D.
Bethesda, Maryland

The human electroretinogram has been studied in response to individual light stimuli (single flash electroretinogram) and intermittent light stimuli (flicker electroretinogram). There is evidence from both types of investigation that the evoked electrical response is a resultant of scotopic ("rod system") and photopic ("cone system") components.

Analysis of the single flash electroretinogram has demonstrated that the positive potential elicited from the human eye may be differentiated into a rapid photopic wave (x-wave) and a slower scotopic wave (b-wave).[1,2] The preceding negative potential (a-wave) can also reflect photopic and scotopic processes.[3-6]

Recent studies[7,8] have been made of flicker fusion frequency in the human electroretinogram (that is, the highest stimulus frequency at which each light flash is accompanied by a discrete wave). Dodt and Wadensten[8] plotted stimulus intensity against flicker fusion frequency and obtained a curve with a sharp change in slope at the higher intensities. As in psychophysical studies of the light intensity-flicker fusion frequency relationship, the low intensity section can be identified with scotopic function and the high intensity section, with photopic function.

The photopic-scotopic analysis of the single flash and flicker electroretinogram suggests that the response patterns of the flicker electroretinogram may be interpreted in terms of the a-, b-, and x-waves of the single flash electroretinogram. Pertinent observations in this regard have been made by Adrian (1945)[1] and Burian (1954)[9] in normal subjects. Bornschein and Schubert (1953)[10] have studied the a-wave and x-wave in the photopic flicker electroretinogram of normal subjects and a congenital night blind subject. However, no systematic approach to the behavior of a-, b-, and x-waves in the human flicker electroretinogram, under different conditions of adaptation and stimulation, has been reported.

The purpose of this paper is to investigate the photopic and scotopic components of the human flicker electroretinogram in different states of adaptation, while changing stimulus intensity and frequency, in a total color blind, a congenital night blind, and normal subjects.

METHODS

A Burian-Allen contact lens electrode led the electroretinographic potentials from the subject's anesthetized cornea. A plastic headband firmly held the reference electrode against the bridge of the nose, and the ground electrode against the forehead.

The electrodes were connected directly to the amplifying system of a direct-writing eight-channel electroencephalograph (Grass, model IIID). The over-all frequency response was found to be constant from 0.5 to 80 cycles/sec. The electroencephalogram filter was used to obtain a steadier baseline. This increased the lower frequency limit to about two cycles/sec., which was still well below the fundamental frequency of the a-, b-, and x-waves examined in this study. The advantages of this Grass electroencephalograph for electroretinographic recordings were detailed by Armington.[11]

The light source was a Photic Stimulator (Grass model PSI), which was used to deliver flashes from one to 80 per second, at a fixed flash duration of about 10 microseconds. The maximal stimulus intensity (setting No. 16) is referred to as intensity

* From the Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

100, and was calculated at 6×10^7 millilamberts. Due to the short duration of the stimulus, the subjective brightness was about four logarithmic units below this figure. Intensity 50 and 25 were obtained by changing the Photic Stimulator settings to No. 8 and No. 4, respectively, and intensities below 25 were produced by neutral filters (Inconel) used with setting No. 4. The electrical output of the Photic Stimulator was checked with a DC voltmeter at intensity 25 (setting No. 4) which was the highest intensity used in the determination of light intensity-flicker fusion frequency curves. There was a gradual decrease at higher frequencies which did not exceed 10 percent at a frequency of 80 per second.

The Photic Stimulator was set into a blackened cylinder, which was equipped with a holder for filters. The front glass of the flash lamp was 40 centimeters from the subject's eye, presenting a stimulus patch of 18 degrees. Grass red and blue filters, supplied for use with the Photic Stimulator, were employed to alter the spectral composition of the light. The blue filter has a peak transmission (about 55 percent) at 450 mμ, with less than one-percent transmission at 570 mμ. The red filter has maximum transmission beyond 650 mμ (about 90 percent) with less than 10-percent transmission below 600 mμ.

The subject's eye was dilated with 10-percent neosynephrine. Dark-adapted electroretinographic recordings were done in complete darkness, after a 15-minute period of dark adaptation. Light-adapted tracings were obtained with a surround illuminance of about three foot-candles, after previous exposure to an everage room illumination of about eight foot-candles.

The right eye of normal subjects, a congenital night-blind (M. G.), and a total color-blind (V. M. M.) were tested in these experiments. Details of the clinical and psychophysical studies in the patients with congenital anomalies are included in a forthcoming publication.[12]

Scotopic flicker electroretinogram

The isolated scotopic flicker electroretinogram can be studied in the dark-adapted totally color-blind subject under all conditions of stimulation, since photopic potentials are completely lacking in this anomaly.[12, 13] In the normal subject, the appearance of photopic responses may be prevented by using stimuli of low intensity. The blue end of the spectrum, to which the photopic system is least sensitive, affords the widest range of stimulus intensities which can be used in the normal subject for eliciting the isolated scotopic flicker electroretinogram.

Figure 1-A shows the flicker electroretinogram of a dark-adapted normal subject with low intensity flashes of blue light at frequencies between one and 16 per second. At all frequencies of stimulation, the first b-wave is the high amplitude response characteristic of the single flash electroretinogram at this level of adaptation and stimulus intensity. The flickering b-waves at low rates of stimulation maintain the same amplitude as the initial response. There is a reduction in size of the waves at each higher rate of stimulation until they cannot be distinguished above baseline irregularities. The frequency at which this occurs is defined as the electroretinographic flicker fusion frequency.

The amplitude relationships of the flickering b-waves follow a predictable pattern. At low flicker frequencies, they are equal in size. As flicker frequency increases, there is a relative depression of the early waves (that is, the waves following the first response), which gradually increase to a steady-state where amplitude remains constant during several minutes of stimulation. With flicker rates approaching fusion frequency, when the steady-state potentials are present but reduced, the greater depression of the early waves results in a period in which no response is recorded. The term "nonflickering period" has been used to describe a similar phenomenon in the cat flicker electroretinogram, and it appears applicable here.[14]

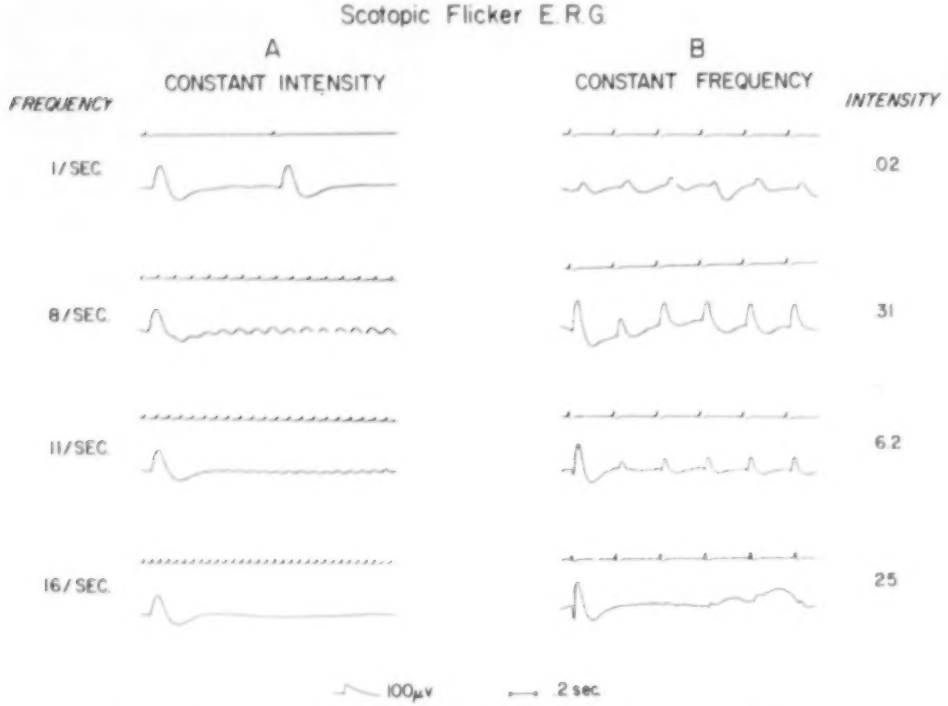

Fig. 1 (Goodman and Iser). The scotopic flicker electroretinogram with increasing frequency at a constant intensity, and increasing intensity at a constant frequency. Column A responses are from a dark-adapted normal subject, with a low intensity blue stimulus. Column B responses are from a dark-adapted color blind subject with white light stimuli, at a frequency of three/sec. Intensity is listed in arbitrary units.

The appearance of the scotopic flicker electroretinogram, elicited with increasing intensities of white light at the same stimulus frequency (three/sec.), is shown in Figure 1-B. This is from the dark-adapted eye of a totally color blind subject. At the lowest intensity, the flickering b-waves are of the same amplitude as the initial response. As intensity is increased, there is a reduction of the flickering potentials relative to the first response. This reduction is greatest for the early flicker waves, and eventually develops into a nonflickering period, as evident with intensity 25. The greater this early depression, the longer the delay in attaining the steady-state characteristic for this stimulus intensity and adaptation state.

The scotopic a-wave is elicited only with the stronger stimuli, but once present it follows the pattern noted for the flickering b-waves. This is evident with intensity 6.2, where the a-wave and b-wave are marked in the first response, absent in the second response, but reappear in small amplitude during succeeding responses.

Results of the determination of flicker fusion frequency with increasing stimulus intensity, in the color blind subject, are plotted graphically in Figure 2. There is a gradual increase of flicker fusion frequency from 12/sec. (intensity 0.02) to 22/sec. (intensity 0.31, 0.6, and 1.2) and then a fall in flicker fusion frequency with higher intensities to about five/sec. at intensity 25.

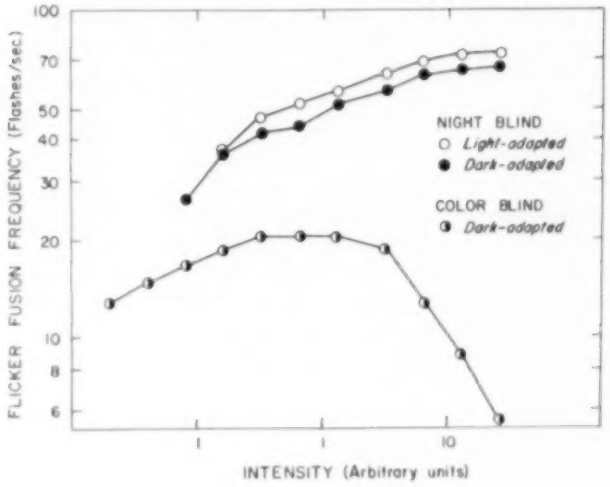

Fig. 2 (Goodman and Iser). The flicker fusion frequency-light intensity relationship in a totally color blind subject (dark-adapted) and a congenitally night blind subject (dark-adapted and light-adapted). No electroretinographic response is elicited in the light-adapted color blind subject. The lower curve is identified with scotopic function, and the two upper curves with photopic function. White light stimuli were used, with intensity listed in arbitrary units.

Interpretation

A striking feature of these studies is the similarity in the changes produced in the scotopic flicker electroretinogram by increasing flicker frequency at a constant stimulus intensity, or increasing stimulus intensity at a constant rate of flicker. In both experiments the flickering b-waves decrease in size relative to the first response. This reduction is greatest in the early responses, and a nonflickering period develops. The scotopic a-wave exhibits a similar behavior, but this is less apparent because of its small size, and the fact that it can be elicited for study only by using high intensity stimuli.

A common factor in the two experiments cited above is the increase in light adaptation which occurs with increasing stimulus frequency or intensity. The effect of light adaptation in raising the scotopic threshold is well known from psychophysical data,[15] and from the decrease in size of the scotopic a-wave and b-wave that it causes in the single flash electroretinogram.* It is probable that the light adaptation produced by increasing the intensity and/or frequency of the flickering stimulus accounts for the reduced amplitude of the flickering a-waves and b-waves relative to the first response.

The selective reduction of the early potentials after the initial b-wave, with a later return to a steady-state amplitude, was first noted by Adrian.[1] He explained this on the basis of a massive discharge of the accumulated reserves of the retina by the first stimulus, with a gradual return to a state of dynamic equilibrium. The present study has extended Adrian's observations on this phenomenon. The early depression may be absent at low stimulus intensity or flicker rate, but it can be elicited if one or both of these variables is sufficiently increased. Furthermore, at high intensities or flicker rates, where all b-waves after the first response are reduced in size, the greater depression of the first few potentials may result in a nonflickering period several seconds in duration. These findings suggest that the delay in return to the steady-state amplitude, attributed by Adrian to the large initial response, may also be influenced by the repetitive light stimuli which occur during the period of readjustment preceding the attainment of the steady-state.

The rise and fall of flicker fusion fre-

* See Bornschein[4] and Auerbach and Burian[6] for discussion and references.

quency in the color-blind subject, with increasing stimulus intensity, is understandable in terms of the paradoxical effect that a rise in stimulus intensity has on the amplitude of the scotopic steady-state potentials. Though the primary effect of a stronger stimulus is to evoke a larger scotopic response, as demonstrated in single flash electroretinographic studies of totally color-blind subjects,[12,13] the repetition of this stimulus causes an increase in light adaptation which acts to diminish the scotopic potentials. During the ascending part of the intensity-flicker fusion frequency curve, the effect of light adaptation is insufficient to prevent an increase in size of the flickering b-waves with each increase in stimulus intensity. This is reflected in Figure 2 where a change in stimulus intensity from 0.02 to 0.31 results in an increase in size of the steady-state waves even though light adaptation is adequate to depress these waves below the amplitude of the first response. During the descending part of the intensity-flicker fusion frequency curve, the stimulus intensities are so marked that light adaptation is sufficient to cause a decrease in size of the flicker potentials with each increase in stimulus intensity. This is demonstrated in Figure 2 by comparing the amplitude of the flickering waves with intensity 0.31, 6.2, and 25.

The electroretinographic flicker fusion frequency-light intensity relationship was studied in a totally color blind subject by Dodt and Wadensten.[8] The maximum flicker fusion frequency was 18/sec., slightly lower than the 22/sec. maximum in this study. A decrease in flicker fusion frequency was noted with the brightest insensities, paralleling the results in the present subject. An analysis of the scotopic flicker electroretinogram was not attempted. A preliminary report by Wadensten[16] of another subject with total color blindness, noted a maximum electroretinographic flicker fusion frequency of 25/sec. The details of the flicker fusion frequency-light intensity curve have not yet been published.

Photopic flicker electroretinogram

The photopic flicker electoretinogram may be isolated in the normal subject under conditions of strong light adaptation, which prevents the appearance of scotopic potentials.

Figure 3 shows the photopic flicker electroretinogram at frequencies from 10 to 60/sec., as obtained from a light-adapted normal subject with high intensity white light flashes. At frequencies below 15/sec. the individual waves are discrete, and the two components of the diphasic single flash photopic response are clearly seen. There is an initial negative potential, the photopic a-wave, followed by a sharp positive wave of short duration, the x-wave. A small positive deflection, of uncertain significance, appears immediately after the x-wave. At a frequency of about 15/sec. the a-wave appears shortly after the return of the descending limb of the x-wave to the baseline. The pattern produced by the negative a-wave, preceded by a brief extent of baseline and the aforementioned positive deflection, is that of a "step" or "hump" which follows the positive x-wave.

With frequencies above 25/sec. the individual diphasic photopic responses (that is, a-wave and x-wave) can no longer be distinguished, but are replaced by a series of sinusoidal waves. This is caused by the appearance of the a-wave *during* the descending limb of the x-wave. The a-wave blends with this part of the x-wave, and transforms a complex response into a sinusoidal one.

The amplitude of the flickering potentials is progressively decreased with stimulus frequencies about 30/sec. and higher, until sinusoidal waves cannot be distinguished above baseline irregularities (fig. 3-F). The frequency at which this occurs is defined as the electroretinographic flicker fusion frequency. However, even at high flicker frequencies, well-defined positive deflections are present at the beginning and end of the flicker electroretinographic record, although the rest of the responses are decreased in size (fig. 3-E and G) or apparently fused

(fig. 3-F). The significance of these findings will be discussed in the section on interpretation.

Flicker electroretinographic records in a congenitally night blind subject, at different intensities and frequencies of stimulation, are included in Figures 4 and 5. They are compared to the photopic flicker electroretinogram of a normal subject under the same conditions. In all records the a-wave in the night blind subject is larger than in the normal subject, so the positive potential which follows does not always reach the baseline. This negative type of response has been noted previously in single flash studies of two other congenitally night blind subjects.[17, 18]

The absence of scotopic function in psychophysical studies of the congenitally night blind subject suggests that the flicker electroretinogram here should be photopic in nature regardless of adaptation or stimulus conditions. Single flash electroretinographic studies in this subject show that the positive potential has the characteristics of the photopic x-wave, but the a-wave surprisingly reveals evidence of scotopic as well as photopic activity.[12] This explains the large size of the initial a-wave when the flicker electroretinogram is recorded in complete darkness (fig.

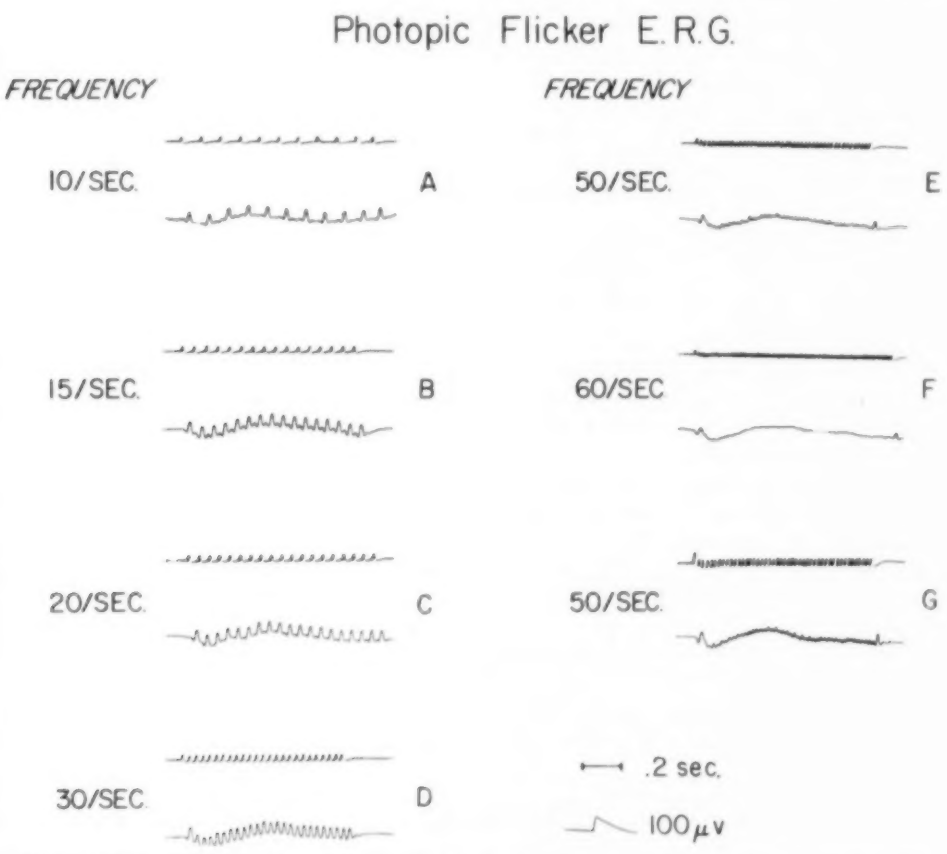

Fig. 3 (Goodman and Iser). The photopic flicker electroretinogram with increasing stimulus frequencies. The records are from a normal subject, in the light-adapted state, with white light flashes of intensity 25 (A-F) and intensity 50 (G).

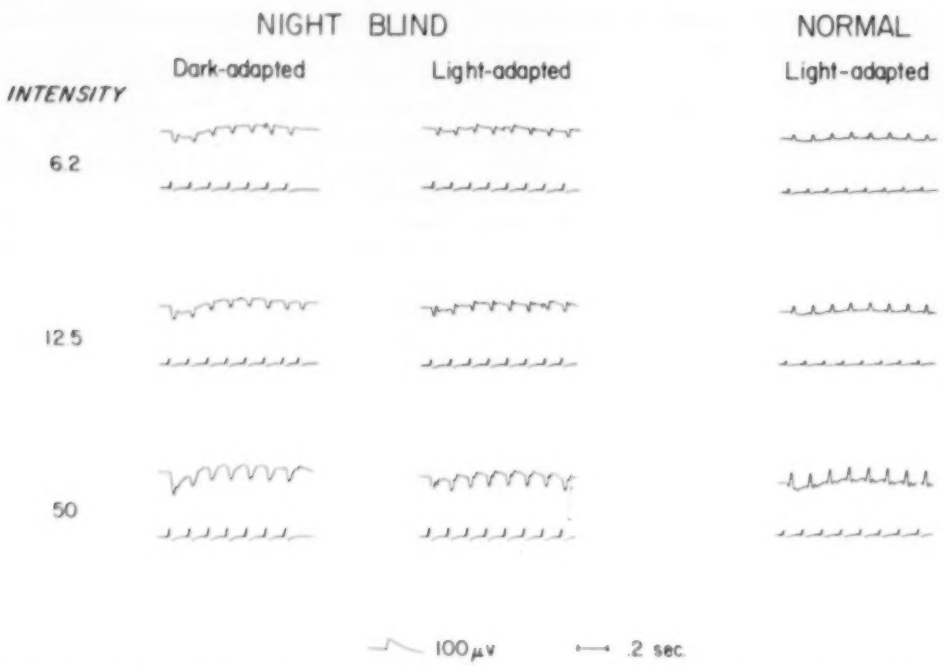

Fig. 4 (Goodman and Iser). Comparative responses from a night blind subject (dark-adapted and light-adapted), and a normal subject (light-adapted), to white light stimuli of increasing intensity, at a constant frequency of eight/sec. The initial a-wave in the dark-adapted night blind subject is larger than its counterpart in the light-adapted night blind subject because of the presence of a scotopic component in the a-wave.

4, dark-adapted*). The light adaptation produced by the flickering light stimuli quickly reduces the size of this a-wave by eliminating the scotopic component.

Another manifestation of the scotopic a-wave component in the dark-adapted electroretinogram of the night blind subject is demonstrated in Figure 5. The first light flash is delivered about 500 milliseconds before the actual flicker electroretinogram is begun. The response to this stimulus is a large a-wave of about 200 microvolts. However, the high stimulus intensity of this flash, with only a short interval before the start of the flickering stimuli, causes sufficient light adaptation to depress the scotopic contribution to the first a-wave of the flicker electroretinogram. As a result the a-wave is reduced to about the same size as in the light-adapted flicker electroretinogram.

Figure 4 compares the response of the night blind and normal subject to increasing stimulus intensity, at a constant flicker rate of eight/sec. The photopic a-wave and x-wave increase in amplitude with each gain in stimulus intensity. The x-wave in the light-adapted state, and the small positive deflections which follow them, are more

* The term "dark-adapted flicker electroretinogram," used in illustrations and text, refers to testing done in complete darkness after preliminary dark adaptation. It should be noted, however, that with the onset of the high intensity flickering stimuli which are necessary to elicit photopic responses, light adaptation rapidly occurs. The term "dark-adapted flicker electroretinogram" is retained, however, to differentiate such an electroretinographic record from that obtained in lighted surrounds after preliminary light adaptation—the "light-adapted flicker electroretinogram."

prominent than those recorded in complete darkness.

Aside from the large initial a-wave response during dark adaptation, the course of the flicker electroretinogram in the night blind subject (fig. 5), obtained in darkness or light adaptation, parallels that of the photopic flicker electroretinogram in normal subjects (fig. 3). At 15/sec. flicker frequency there is a "step" or double-peaked wave in the night blind flicker electroretinogram. As in the normal subject, that "step" represents the baseline, or a small positive deflection riding on the baseline. It is higher in the night blind subject because the baseline is above the peak of the x-wave. Sinusoidal potentials appear at a frequency of about 25/sec., which progressively decrease in size with increasing rates of flicker.

The presence of a large positive deflection at the beginning and end of the flicker electroretinogram with high frequencies, relative to the small size of the flickering potentials, is apparent in the night blind as well as in normal records. The night blind flicker electroretinogram, however, always begins well below the baseline, in keeping with the large amplitude of the negative a-wave deflection noted in the single flash electroretinogram. In contrast, the a-wave in the normal subject is small and the x-wave projects clearly above the baseline, producing a predominantly positive flicker electroretinographic response.

The flicker fusion frequency at different stimulus intensities is plotted graphically for the night blind subject in the dark-adapted and light-adapted state (fig. 2). Both curves parallel each other in their shape and course, showing an increase in flicker fusion frequency with each rise in stimulus intensity. The flicker fusion frequency obtained in the light-adapted state, however, tends to be slightly higher.

With the maximum light intensity used in this experiment, flicker fusion frequency is

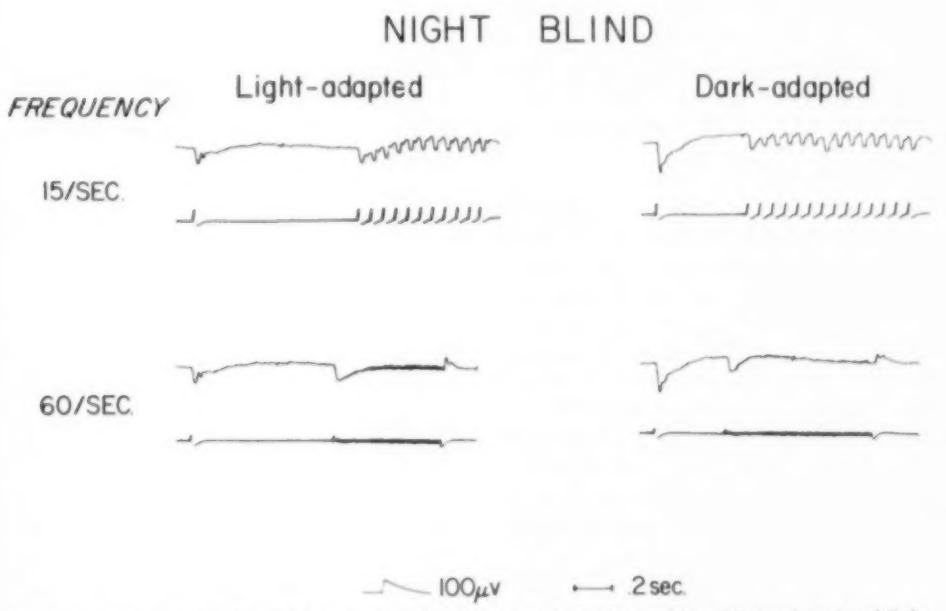

Fig. 5 (Goodman and Iser). Flicker electroretinogram in a night blind subject (dark-adapted and light-adapted) preceded by a single flash response. White light flashes, intensity 25.

65/sec. (dark-adapted) and 69/sec. (light-adapted). A similar curve is obtained in the light-adapted normal subject (fig. 8).

Interpretation

The temporal characteristics of the photopic response are an important factor in determining the photopic flicker electroretinogram patterns and flicker fusion frequency. The short implicit time* and rapid course of the x-wave and a-wave account for the discreteness of these waves up to frequencies of about 20/sec.

From 20 to 30/sec. the a-wave blends with the descending branch of the x-wave, while the positive response maintains approximately the same amplitude as separate x-waves at lower frequencies. With frequencies above about 30/sec., the a-wave occurs during the ascending limb of the x-wave, and exerts a dual effect on the response. It "cuts off" the x-wave before the full development of this positive wave is reached, resulting in a response with a shorter implicit time, but smaller amplitude. The decrease in amplitude is a limiting factor in the attainment of maximal flicker fusion frequencies, since the flickering potentials become too small to distinguish from baseline irregularities.

Within the range of stimulus intensities used in the present study, each increase in intensity produces a rise in flicker fusion frequency. This appears to be the result of a gain in amplitude of the flicker waves, which are therefore more easily differentiated at higher flicker frequencies (compare fig. 3-E and G). It is possible that the photopic response with stronger intensities may also have a shorter temporal course, which could be an added factor in producing a discrete response at high frequencies, but the present recording apparatus was not suitable for such fine measurements.

The interpretation of the photopic flicker electroretinogram presented here suggests that the first x-wave response at high flicker frequencies should be reduced in size by the next a-wave, and therefore it should be of approximately the same size as the flickering potentials which follow. This is not the case, as evident in Figure 3-E and G. The large amplitude of this first response relative to the amplitude of those that follow is similar to observations in the scotopic flicker electroretinogram.

As noted previously, the first b-wave response in the scotopic flicker electroretinogram is what would be expected in a single flash response, even when stimuli of high frequency appear during the initial discharge —stimuli which are sufficient to eliminate scotopic response in the remainder of the record (see fig. 6-A, 30/sec. flicker). Furthermore, in the mixed flicker electroretinogram which will be discussed in the next section, the initial b-wave persists even when x-waves are superimposed upon it, and the rest of the flicker record is a photopic one (see fig. 6-B, 30/sec.).

These findings in the scotopic and photopic flicker electroretinogram suggest that the first discharge tends to maintain its integrity despite the presence of factors which are sufficient to reduce the size of the potentials which follow.

The interpretation of the development of the photopic flicker electroretinogram in terms of interference between a-wave and x-wave, as presented in the previous paragraphs, is based upon the studies of Bornschein and Schubert.[10] An interesting phenomenon which they demonstrated at high flicker frequencies was the large size of the last positive potential relative to the preceding flickering potentials. This is also evident in the flicker electroretinogram of the normal subject (fig. 3-E and G) and night blind subject (fig. 5, 60/sec.) in the present study.

Since this large positive deflection was in response to the last flickering stimulus, they suggested that its size was due to the fact that there was no succeeding a-wave which could "cut off" the preceding positive re-

* Interval between onset of stimulus and peak of wave.

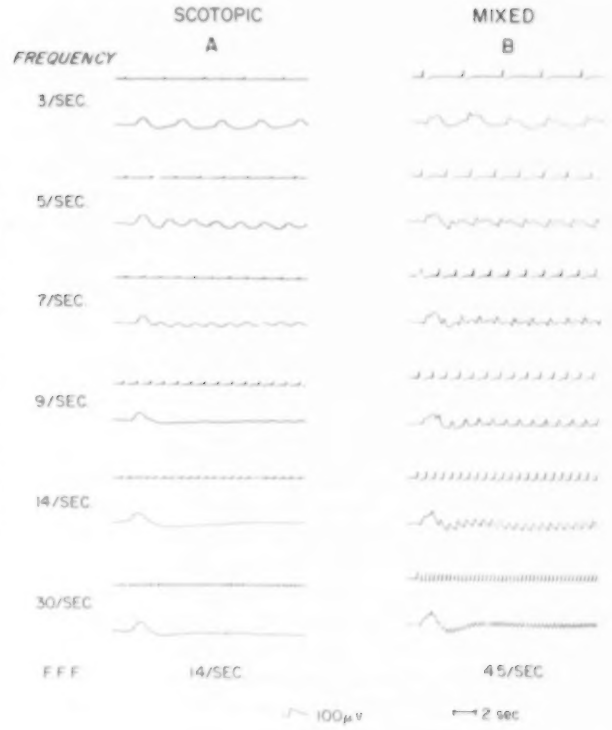

Fig. 6 (Goodman and Iser). A comparison between the scotopic flicker electroretinogram and mixed flicker electroretinogram in a normal subject. A low intensity blue stimulus was used to elicit the scotopic electroretinogram, and a red stimulus for the mixed electroretinogram. The response to red light at low frequencies shows the characteristic separation between x-wave and b-wave. The stimuli were chosen so that the blue and red flashes would have approximately equal effectivity for the scotopic system. (Blue filter used with intensity 0.08. Red filter used with intensity 100).

sponse. If this last positive response is an x-wave, as its appearance and implicit time suggest, then this explanation is in keeping with the concept that high intensity photopic flicker electroretinogram patterns are the result of a-wave and x-wave interference. However, as Bornschein and Schubert have noted, the possibility that an "off-effect" may contribute to this last positive response cannot be excluded.

The effect of increasing stimulus intensity on the photopic and scotopic flicker electroretinogram affords an interesting comparison. In the case of the photopic flicker electroretinogram, a rise in stimulus intensity causes an increase in size of the flickering waves (fig. 4), and a higher flicker fusion frequency (fig. 2). This happens despite the marked light adaptation which occurs when the strong intensity light stimuli, which are necessary to elicit the photopic potentials, are flickered at rates as high as 60/sec. However, the role of light adaptation as the major determinant of amplitude and flicker fusion frequency is evident in the scotopic flicker electroretinogram. Here, there is a gain in amplitude of the flickering potentials, and a rise in flicker fusion frequency, only when the increase in stimulus intensity is made within a limited range of low intensity stimuli (figs. 1 and 2). The use of high intensity stimuli markedly depresses the flickering scotopic a-waves and b-waves, and the scotopic flicker fusion frequency.

The flicker electroretinogram records in the night blind subject suggest that light adaptation may possibly augment the photopic response. The flicker fusion frequency-light intensity curve shows somewhat higher values of flicker fusion frequency in the light adapted state compared to testing done in dark adaptation. This enhancement of photopic response by light adaptation, however, is not peculiar to the flicker electro-

retinogram. Dark adaptation studies of the x-wave in this night blind patient, with the single flash electroretinogram, revealed a decrease in amplitude during the early part of dark adaptation.[12] A similar observation was noted by Armington[19] in normal subjects, using red light to elicit the x-wave response. Burian[9] has shown that the x-wave in normal subjects, after prolonged dark adaptation, increases during the first few minutes of light adaptation, and this rise may be accelerated by light stimulation. These results are difficult to correlate with psychophysical studies of photopic function, where a definite increase in photopic sensitivity occurs during dark adaptation, which is decreased by light adaptation.

Though there are several reports[17,19,20] concerning the single flash electroretinogram in congenitally night blind subjects, the flicker electro-retinogram has been studied only by Bornschein and Schubert.[10] Using a light stimulus of a fixed high intensity, they observed a flicker fusion frequency of about 60/sec., which was within the range of three normal subjects tested under the same conditions. Their interpretation of the photopic flicker electroretinogram in terms of a-wave and x-wave was applicable to the present study, in which testing was extended to varying adaptation states, a wider range of stimulus intensities, and a light flash of markedly different temporal characteristics. The results of this study, therefore, afford additional support for the analysis of the photopic flicker electroretinogram in terms of single flash components.

The fact that the photopic flicker electroretinogram is derived from the photopic single flash response is of importance for clinical flicker electroretinography. A recent clinical electroretinographic investigation concluded that the high intensity flicker electroretinogram gives information concerning the photopic component unobtainable by single flash methods.[8] This is true for the dark-adapted single flash response to white light, which is routinely used in clinical electroretinographic studies. The b-wave predominates in such a tracing, and the small x-wave which is buried within the scotopic response cannot be isolated for measurement. However, a single high intensity stimulus in the light-adapted state will elicit the isolated photopic a-wave and x-wave, which can be measured as an index of photopic function. If the single flash photopic response is depressed or absent, then the photopic flicker electroretinogram should be similarly affected, since it is based upon the single flash response. This has been confirmed in clinical studies.[21]

Mixed flicker electroretinogram

The mixed flicker electroretinogram is an electroretinogram in which scotopic and photopic flickering potentials are present in the same tracing. It may be obtained from normal subjects in the dark-adapted state, at frequencies of flicker within the scotopic range, by using stimuli of sufficient intensity to elicit photopic, in addition to scotopic, components.

Figure 6 demonstrates the flicker electroretinogram in a normal dark-adapted subject with blue and red stimuli. The red stimulus was chosen because it is known to elicit a response with the clearest separation of the rapid x-wave and slower b-wave.[1] A blue stimulus was used at an intensity which produced a b-wave of approximately the same amplitude as the red stimulus. The low intensity of this blue stimulus, and the insensitivity of the photopic system for light of short wavelength, explain the lack of a photopic component. Both stimuli, therefore, have equal effectivity for the scotopic system, but only the red flashes evoke a photopic response.

The response to the blue stimulus illustrates a typical scotopic flicker electroretinogram. As flicker frequencies are increased, there is a depression of the flickering potentials, the development of a nonflickering period (nine/sec.) and a flicker fusion frequency of about 14/sec. With the red stimulus, a mixed flicker electroretinogram is evident with the lower frequencies. The x-

wave and b-wave are seen in their full extent at three and five/sec. flicker. The x-wave, preceded by the photopic a-wave, encroaches on the b-wave with seven and nine/sec. flicker, and only the beginning of the ascending limb of the b-wave is seen before the photopic a-wave begins. From 14/sec. flicker until a flicker fusion frequency of about 45/sec. the response to this red stimulus is typical of the photopic electroretinogram, except for the persistence of a large b-wave in the first response.

The behavior of the b-wave in the mixed flicker electroretinogram elicited with red light is similar to its behavior in the pure scotopic flicker electroretinogram elicited with blue light. As flicker is increased from three to nine/sec., there is a progressive depression of the flickering b-waves relative to the first response in both the mixed and scotopic flicker electroretinogram. No further comparisons can be made beyond nine/sec. since the b-wave response cannot be distinguished in the tracing.

The appearance of the photopic components in the mixed flicker electroretinogram is also consistent with observations in the isolated photopic electroretinogram. At a low flicker frequency, the x-waves tend to be of equal amplitude. There is a "step" following the x-waves at 14/sec., a sinusoidal pattern at about 30/sec., and a progressive reduction in size of the flickering waves with increasing frequency. Fusion occurs at 45/sec., which is within the photopic flicker fusion frequency range (fig. 2). Even when scotopic potentials are elicited in the mixed flicker electroretinogram, the x-waves are still clearly present, as might be predicted from their appearance in the photopic flicker electroretinogram. This is well demonstrated in the first response to red light flicker at 20 and 30/sec. where the x-waves may be seen riding the ascending and descending limbs of the scotopic b-wave.

The response to white light flicker at two different intensities, in a normal dark adapted subject, is shown in Figure 7. (The x-waves and b-waves in these records, as in the single flash response to white light, show

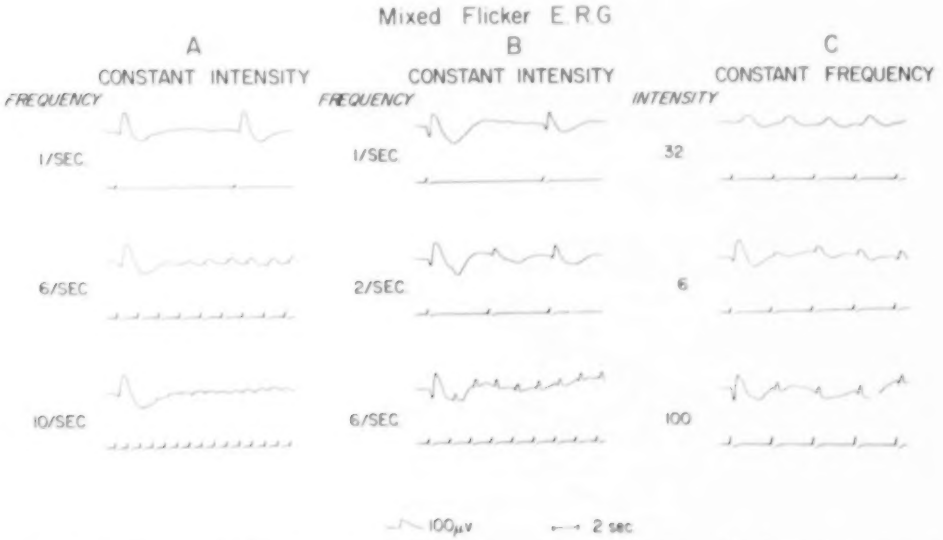

Fig. 7 (Goodman and Iser). The mixed flicker electroretinogram with increasing frequency at a constant intensity (A and B) and increasing intensity at a constant frequency (C). Records are from a dark-adapted normal subject, with white light stimuli. The mixed electroretinogram responses with white light do not show the clear separation between x-waves and b-waves noted previously with red light (fig. 6-B). Constant intensity in A was 0.6, and in B 12.5. Constant frequency in C was three/sec.

less separation than with red stimuli.)

Figure 7-A demonstrates the mixed flicker electroretinogram with a low intensity stimulus. At a frequency of one/sec. the b-wave predominates in the response, and the x-wave cannot be separated. With a frequency of six/sec. the flickering b-wave potentials are depressed, with a greater depression in the first few responses. As a result, the x-wave can now be distinguished as the first peak of a double-peaked response. It appears to grow larger in subsequent responses, just as the b-wave grows larger, because it is riding on the ascending limb of the b-wave. With flicker at 10/sec. this pattern is repeated, but the b-waves show a greater reduction in size, and the x-wave is therefore distinguished more easily. During the first few responses it is actually larger than the b-wave, but this situation is reversed by about the seventh response. The response to a higher intensity flickering stimulus (fig. 7-B) shows a similar pattern, with a more dramatic separation of photopic and scotopic components. The stronger stimulus produces an x-wave of larger size, and the b-wave is more quickly reduced in amplitude by the light adaptation which the intense flickering stimulus produces. Thus, at one/sec. flicker, the second b-wave is already reduced in size, and with six/sec. flicker, the b-wave is evident only with the first response.

The behavior of the x- and b-waves, at a constant flicker rate and increasing intensity, is demonstrated in Figure 7-C. With intensity 0.32 the response consists of low amplitude waves, about equal in size. It is doubtful whether an x-wave is present at this low intensity. With intensity 6, the b-waves are reduced in size and the selective depression of the first scotopic flicker response allows the x-wave to project above the level of the b-wave. The flicker electroretinogram at intensity 100 shows a large b-wave in the first response which is no longer evident with subsequent stimuli. The flickering potentials at this high intensity are composed of x-waves, and appear to maintain about the same amplitude throughout the response.

The flicker fusion frequency-light intensity curve of a normal dark-adapted subject is shown in Figure 8. There is a sharp change in slope at intensity 0.62, which divides the curve into two sections. The range of flicker fusion frequency in the low intensity section is 11 to 17/sec., and in the high intensity section—about 30 to 65/sec. Testing in the light-adapted state results only in the high intensity section of the curve.

Interpretation

The mixed flicker electroretinogram has been studied in response to red and white stimuli at different frequencies and con-

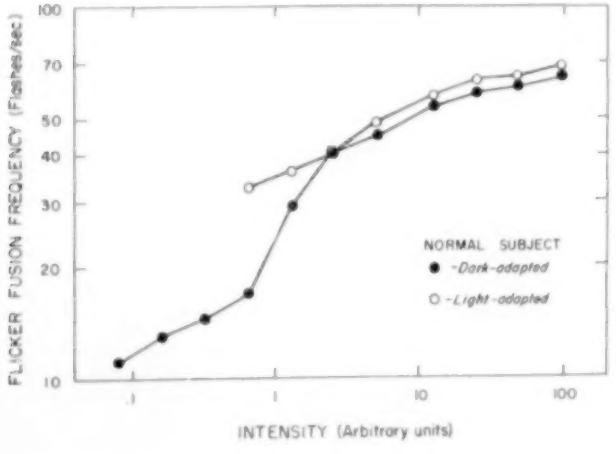

Fig. 8 (Goodman and Iser). The flicker fusion frequency-light intensity curve in a normal subject (dark-adapted and light-adapted). In the dark-adapted state, a curve with two branches is obtained. Only the high intensity (photopic) section is present in the light-adapted state. White light stimuli were used, with intensity listed in arbitrary units.

stant intensity, and to white light at a constant flicker rate but with changing stimulus intensities.

The depression of the flickering b-waves with increasing intensity at a constant flicker rate, or increasing flicker rate at a constant intensity, is apparent in the mixed flicker electroretinogram, as in the scotopic flicker electroretinogram. These observations are also in keeping with the known effects of adaptation and intensity on the single flash scotopic electroretinogram, as discussed previously.

The photopic component of the mixed flicker electroretinogram also follows the course described for the isolated photopic flicker electroretinogram. With a constant flicker rate and increasing intensity, there is an increase in amplitude of the x-waves, as would be expected from intensity curve studies of the x-wave in the single flash photopic flicker electroretinogram.[22] Scotopic potentials can be elicited in the mixed flicker electroretinogram below 15 to 20/sec. Since the x-wave rides on the b-wave at these low rates of flicker, it may appear to vary with the amplitude of the b-wave. However, as soon as intensity and/or flicker rate are increased sufficiently to eliminate the scotopic component from the record, the x-wave is seen to be following its normal flicker course.

The flicker fusion frequency-light intensity curve of the mixed flicker electroretinogram shows a low and high intensity portion, confirming the observations of Dodt and Wadensten[8] (fig. 8). The form of this curve is a composite of the flicker fusion frequency-light intensity functions described in the present study for the color blind subject (scotopic flicker electroretinogram—low intensity section of curve), and the night blind subject (photopic flicker electroretinogram—high intensity section of curve). The low intensity (scotopic) section may be eliminated in the normal subject by testing in the light-adapted state.

The scotopic and photopic components appear to pursue independent courses in the mixed flicker electroretinogram, producing a response which is a simple resultant of the isolated scotopic and photopic flicker electroretinogram. The behavior of the a-, b-, and x-wave in the mixed flicker electroretinogram, just as in the scotopic and photopic flicker electroretinogram, is consistent with previous single flash studies.

Summary

The purpose of this paper was to investigate the photopic and scotopic components of the human flicker electroretinogram in different states of adaptation, while changing the intensity and frequency of the light stimulus.

The scotopic ("rod system") flicker electroretinogram was studied in normal and totally color blind subjects. The flickering positive potentials (b-waves) decreased in amplitude with increasing rates of stimulation and/or stimulus intensity. A maximum flicker fusion frequency of 22/sec. was obtained with light stimuli of moderate intensity, which decreased with stimuli of higher intensity. These effects were due to the influence of light adaptation, produced by the repetitive light stimuli, on the scotopic apparatus.

The photopic ("cone system") flicker electroretinogram was studied in normal and congenitally night blind subjects. The complex response patterns were interpreted in terms of the interference of the negative (a-wave) and positive (x-wave) components of the single flash photopic electroretinogram. Flicker fusion frequency increased with each increment in stimulus intensity, in a range from about 28 to 70/sec.

Under conditions where both photopic and scotopic components were present (mixed flicker electroretinogram), the behavior of these components proceeded independently, as demonstrated in the isolated photopic and scotopic flicker electroretinogram. The flicker fusion frequency-light intensity curve showed two branches, which was a composite of the curves obtained in the totally color blind and congenitally night blind subjects.

The response of the a-, b-, and x-waves in the flicker electroretinogram to changing adaptation and stimulus intensity, were consistent with the behavior of these waves in previous studies of the single flash electroretinogram.

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We are greatly indebted to Dr. Hans Bornschein (University of Vienna), visiting scientist at the Ophthalmology Branch, for his advice and suggestions throughout this investigation.

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CLINICAL STUDIES WITH FLICKER ELECTRORETINOGRAPHY*

GILBERT ISER, M.D., AND GEORGE GOODMAN, M.D.
Bethesda, Maryland

INTRODUCTION

Ophthalmologists have long sought more accurate means of testing retinal function, especially in the presence of partially opaque media. With the development of the corneal contact lens electrode by Riggs[1] in 1941, and Karpe[2] in 1945, electroretinography, a procedure previously only a tool for animal investigation, took its place in human physiologic study. The answer to the search for an objective test of retinal function appeared to lie in the electroretinogram.

In the past 10 years clinical studies have concentrated mainly on the scotopic portion of the electroretinogram or b-wave, after the technique of Karpe. This consists of the study of the potential evoked in the dark-adapted eye by a light stimulus of moderate intensity.

Although the photopic component of the electroretinogram, the potential isolated in the light-adapted eye, was described in 1945 by Adrian,[3] its analysis has not as yet become part of routine clinical electroretinography. More recently Dodt[4] and Dodt and Wadensten[5] have reported on the results of their studies of photopic flicker using high intensity light stimuli. With this method of flickering the photopic responses, a clearer picture of this neglected component emerges. The amplitude of the response at various frequencies can be measured, and the flicker fusion frequency can be determined.

The scotopic electroretinogram has been found to have definite limitations as a diagnostic aid, especially in diseases involving the macula specifically, or cone (photopic) system in general.[6] With the development of better methods for recording the photopic electroretinogram, it became of great interest to evaluate its possibilities in clinical investigation.

The present study was undertaken to determine whether the photopic electroretinogram, as reflected by the single flash and flicker techniques would provide more diagnostic information than the standard single flash scotopic electroretinogram in retinal disease.

METHODS

The testing was done with the patient at rest on a hospital bed. The stimulating light was centered so that both eyes were stimulated equally. Simultaneous recordings were made from both eyes using Burian-Allen contact lens electrodes, an indifferent electrode placed on the bridge of the nose, and a Grass electroencephalograph. The patient was grounded through the electroencephalograph by electrodes on the forehead (fig. 1).

Employing a direct writing eight-channel electroencephalograph, allowed the use of varying gains and time constants in each record. However, measurements of amplitude and flicker fusion frequency were made with the electroencephalograph filter which has been shown to give an undistorted recording of the electroretinogram.[7]

The Grass photic stimulator was used as the light source. This makes use of a xenon tube with a flash of about 10 microseconds' duration, and variable intensity and frequency. The energy distribution of the lamp without filters is above 90 percent from 400 to 575 millimicrons, and then drops off to a low of about 70 percent at about 675 millimicrons. The peak luminance, calculated on the basis of figures supplied by the manufacturer, is about 6×10^7 millilamberts at intensity 16. (The intensity of the flash is calibrated in five steps, 1-2-4-8-16, each of which is $2\times$ the preceding.) Due to the

* From the Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

Fig. 1 (Iser and Goodman). Patient with electrodes in place. Burian-Allen contact lens electrodes on each eye. The indifferent and ground electrodes are incorporated into a plastic holder.

extremely short duration of the flash, the subjective brightness is about four log units below this. Features in the construction of the lamp housing account for a variation of the luminance at different distances, and limitations of the circuits cause a decrease in intensity at high frequencies.

The patients were tested under conditions of both light and dark adaptation. The photopic electroretinogram was taken in a room with background illumination of 15 f.c. after 10 minutes of preadaptation to this light. Scotopic recording was carried out in complete darkness after 10 minutes of dark adaptation.

Although preliminary studies were made with a wide range of stimuli, on the basis of these results an abbreviated standardized technique was adopted. In the light-adapted state, single flash and flicker testing were done using high intensity stimulation. In some of the cases this was followed by testing the response to red light.* Following this, the patient was dark adapted and the response to single flash stimuli of moderate intensity was recorded. The stimulus arrangement for each of these tests was as follows:

Test	Lamp to patient distance	Stimulator setting
Photopic single flash	1 ft.	16
Photopic flicker	1 ft.	8
Red single flash and flicker	1 ft.	16
Scotopic single flash	4 ft.	16

Subjects for this series were drawn from patients referred to the Ophthalmology Branch, National Institute of Neurological Diseases and Blindness. The results of testing 25 normal and 50 pathologic eyes are included in this report. The following ocular diseases are among those represented: isolated macular lesions, peripheral chorioretinitis (without macular involvement), vascular retinopathies, retinal detachment, retinal abiotrophies.

All patients had routine ophthalmologic examination including visual acuity determination, slitlamp examination, and funduscopy. Where indicated, supplementary studies such as perimetry and adaptometry were done.

Of the various measurements made of each record, four have been selected for tabulation. They are: (fig. 2—normal eye):

1. *The photopic single flash or x-wave.* This is the positive component of the response of the light-adapted eye to a stimulus of high intensity. Under the usual conditions of clinical electroretinography (that is, testing the dark-adapted eye), the smaller x-wave is masked by the large b-wave. The combination of low amplitude and the need for high intensity stimulus probably account for the fact that the x-wave has not been more extensively studied in clinical investigation.

2. *Photopic flicker—amplitude at 30/sec.* Photopic flicker is the response of the light-

* The red stimulus was obtained by using color filter No. 1 as supplied by the Grass Company for the Photic Stimulator. This filter has maximum transmission above 650 millimicrons, with less than 10-percent transmission below 600 mμ.

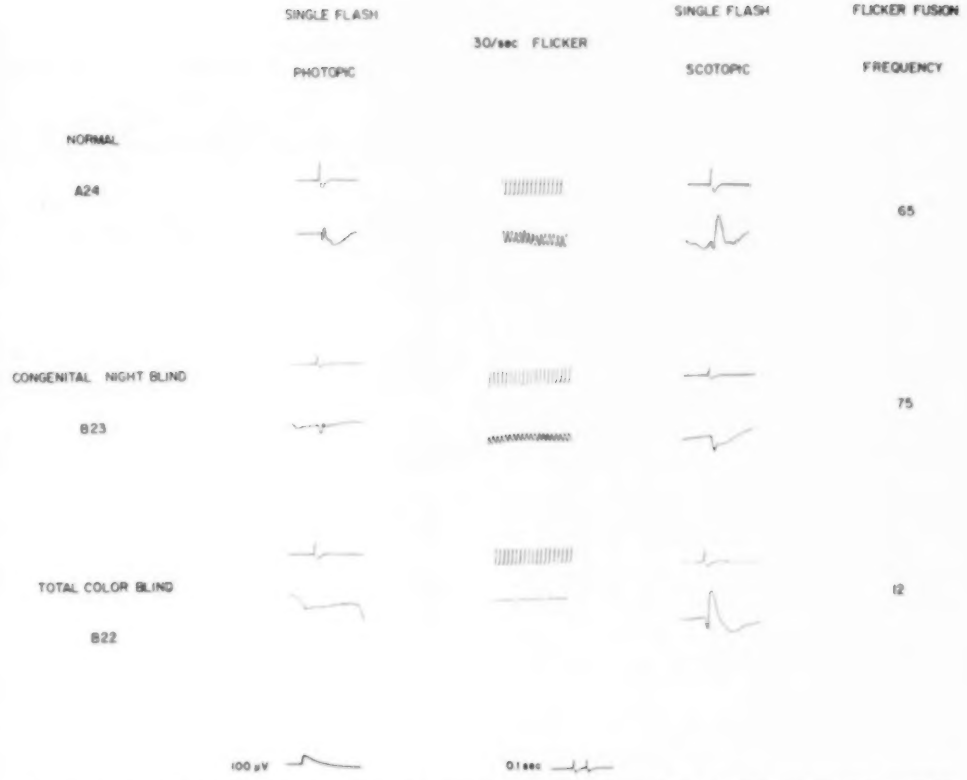

Fig. 2 (Iser and Goodman). The electroretinogram in a normal, congenitally night blind and totally color blind subject. (The identifying numbers refer to listings in Tables A-B-C.)

adapted retina to high intensity flickering light stimuli. Measurement of the amplitude of the flicker wave is a new idea in clinical electroretinography. The flicker wave at 30/sec. is an excellent indicator of photopic function.[5, 9] At this adaptation level all of the scotopic response has been eliminated.

3. *Photopic flicker fusion frequency.* In this study the flicker fusion frequency is defined as the frequency at which the retina is observed to respond to less than half of the high intensity flicker stimuli.

4. *The scotopic single flash or b-wave.* This is the positive component of the response of the dark-adapted eye to a light flash of low intensity. At the stimulus intensity used in this study, the response is a composite of both the b-wave and x-wave. The b-wave, however, greatly overshadows the x-wave which in some of the tracings can be seen as a "hump" in the larger response.

An evaluation of the negative initial components of both the photopic and scotopic single flash response, the a-wave, is not included in this report.

Results

The cases have been tabulated so that they will best illustrate the relative merits of the photopic and scotopic electroretinographic techniques.

1. Normal eyes with normal electroretinogram.

2. Pathologic eyes with abnormal electroretinogram.

3. Pathologic eyes with normal electroretinogram.

TABLE A

NORMAL EYES WITH NORMAL ELECTRORETINOGRAM

Eye	x-wave Amplitude (microvolts)	30/Sec. Flicker Amplitude (microvolts)	Flicker Fusion Frequency	b-wave Amplitude (microvolts)	Other Eye Table No.
1	100	*	65	250	C-11
2	125	*	70	275	C-3
3	125	*	75	300	A-4
4	125	*	70	275	A-3
5	125	*	65	260	B-11
6	*	*	55	390	B-4
7	200	*	55	335	B-12
8	125	*	55+	400	B-13
9	100	100	70	250	A-10
10	100	100	70	300	A-9
11	150	175	75	300	B-14
12	125	100	65	375	B-8
13	150	125	70	325	A-14
14	150	125	*	350	A-13
15	125	125	60+	500	B-9
16	100	100	65	300	B-15
17	175	155	55	400	C-8
18	125	100	65	250	—
19	100	100	60	350	C-16
20	150	125	65	400	B-24
21	150	175	65	450	C-19
22	175	175	65	500	C-22
23	125	125	70+	350	C-23
24	135	125	65	350	B-27
25	150	175	60	350	—

* Not measured.

NORMAL EYES WITH NORMAL ELECTRORETINOGRAM

This group consists of 25 eyes (table A). In some cases normal volunteers are used for controls, while in other instances the normal eye of a patient with uniocular disease is used. In these latter cases the electroretinograms are cross-referenced so that both eyes of a single patient may be compared. Where this type of comparison is possible, each patient may be used as his own control.

Under the present experimental conditions, the normal variation of the photopic and scotopic components is as follows:

Amplitude of photopic single flash or x-wave	100-200 μv
Amplitude of photopic flicker at 30/sec.	100-175 μv
Photopic flicker fusion frequency	55-75/sec.
Amplitude of scotopic single flash or b-wave	250-500 μv

PATHOLOGIC EYES WITH ABNORMAL ELECTRORETINOGRAM

Table B contains the results of 27 eyes with abnormal electroretinograms. They are caused by a variety of diseases: uveitis, retinal detachment, vascular disease, and retinal degenerations. Also included are a congenitally night blind and totally color blind subject. These latter two cases* have been reported on more fully elsewhere.[10]

Except for the congenital retinal abnormalities, there is surprisingly little dissociation between the photopic and scotopic responses. In a few of the tracings there is either borderline agreement or perhaps slight depression of one of the photopic components, but in only one eye is there any real unexplained dissociation (table B, no. 3).

* Kindly referred by Dr. Louise Sloan, Wilmer Institute.

TABLE B

PATHOLOGIC EYES WITH ABNORMAL ELECTRORETINOGRAM

Eye	a-wave Amplitude (microvolts)	30/Sec. Flicker Amplitude (microvolts)	Flicker Fusion Frequency	b-wave Amplitude (microvolts)	Diagnoses	Other Eye Table No.
1	75	75	60	390	Posterior Pole and Macular Chorioretinitis	—
2	100	75	55	125	Chorioretinitis with Multiple Lesions	C-4
3	135	125	60	180	Macular Scar	C-7
4	100	75	45	250	Chorioretinitis—Cloudy Vitreous	A-6
5	25	*	30	50	Myopic & Arteriosclerotic Retinal Degeneration	B-6
6	25	*	30	150	Myopic & Arteriosclerotic Retinal Degeneration	B-5
7	80	40	45	100	Blind Eye—Cloudy Vitreous	—
8	0	0	0	0	Central Vein Thrombosis Secondary Glaucoma	A-12
9	80	75	55	250	Central Artery Occlusion	A-15
10	50	27	45	40	Diabetic Retinopathy	—
11	0	0	0	0	Retinal Detachment	A-5
12	55	*	50	135	Retinal Detachment	A-7
13	90	*	50	200	Retinal Detachment	A-8
14	0	0	0	0	Intraocular Tumor Complete Detachment	A-11
15	40	25	40	100	Intraocular Tumor Retinal Detachment	A-16
16	0	0	0	250	Incomplete Color Blind	B-17
17	0	0	0	250	Incomplete Color Blind	B-16
18	25	25	40	150	Retinal Degeneration	B-19
19	25	25	40	125	Retinal Degeneration	B-18
20	75	75	45	125	Tapetoretinal Degeneration	B-21
21	75	75	45	125	Tapetoretinal Degeneration	B-20
22	0	0	12	425	Total Color Blind	—
23	125	100	75	0	Congenital Night Blind	—
24	75	75	45	125	Chorioretinitis—Dense Vitreous Opacities	A-20
25	50	50	40	50	Optic Neuritis and Retinitis	B-26
26	50	50	40	50	Optic Neuritis and Retinitis	B-25
27	75	55	55	150	Chorioretinitis—Posterior Pole	A-24

* Not measured.

PATHOLOGIC EYES WITH NORMAL ELECTRORETINOGRAM

This group consists of 23 eyes (table C). In all these cases both the scotopic single flash response and the photopic single flash and flicker are within the normal limits as established in the control series. The data as tabulated are from eyes with cataract, optic atrophy, glaucoma, and lesions of the macula.

In addition the eyes with macular disease were tested with high intensity red light stimuli (fig. 4). These results are tabulated in Table D, along with seven normal eyes tested under the same conditions. The responses to the red stimuli are generally slightly lower than to the white, but none

TABLE C

PATHOLOGIC EYES WITH NORMAL ELECTRORETINOGRAM

Eye	x-wave Amplitude (microvolts)	30/Sec. Flicker Amplitude (microvolts)	Flicker Fusion Frequency	b-wave Amplitude (microvolts)	Diagnoses	Other Eye Table No.
1	200	150	65	300	Cataract	C-2
2	200	100	65	350	Cataract	C-1
3	125	*	65	275	Macular Scar	A-2
4	150	110	60	275	Peripheral & Macular Chorioretinal Scars	B-2
5	125	125	55	425	Macular Heredodegeneration	C-6
6	165	165	55	425	Macular Heredodegeneration	C-5
7	185	200	70	350	Macular Scar	B-3
8	150	155	60	375	Macular Chorioretinitis	A-17
9	100	125	70	250	Optic Atrophy	C-10
10	100	125	70	250	Optic Atrophy	C-9
11	100	*	60	275	Eales Disease	A-1
12	100	100	70	350	S-C Hemoglobin Retinopathy	C-13
13	125	75	60	350	S-C Hemoglobin Retinopathy	C-12
14	150	175	75	500	Macular Chorioretinitis	C-15
15	175	175	75	500	Macular Chorioretinitis	C-14
16	125	150	60	325	Macular Chorioretinitis	A-19
17	100	100	60	250	Constricted Visual Fields ?Glaucoma	C-18
18	100	100	60	250	Constricted Visual Fields ?Glaucoma	C-17
19	175	150	65	400	Chorioretinitis	A-21
20	125	160	*	375	Macular Hole	C-21
21	125	100	*	375	Chorioretinitis	C-20
22	225	175	65	500	Macular Chorioretinitis	A-22
23	125	100	65	375	Amblyopia—?Etiology	A-23

* Not measured.

of the differences appear significant from a diagnostic point of view.

In several of the electroretinograms of pathologic eyes a rather unusual qualitative change not previously described is observed in the flicker records. At flicker frequencies of up to 35/sec. a retinal flicker response is obtained with each light flash. However, from approximately 40/sec. to the point of fusion, responses are observed only to alternate light flashes (fig. 3). This phenomenon, which has not been seen in normal eyes, may be another indication of poor retinal condition, but the specific cause is as yet obscure.

DISCUSSION

A. COMPARATIVE VALUE OF THE PHOTOPIC AND SCOTOPIC ELECTRORETINOGRAM

The results of this study indicate that in conditions such as congenital anomalies and hereditary degenerations analysis of the x-wave amplitudes, flicker wave amplitude, and flicker fusion frequencies may add considerably to the information gained from the scotopic electroretinogram alone. However, ocular pathology such as vascular retinopathies, retinal detachments, and chorioretinitis, when they are severe enough to affect the electroretinogram, appear to produce depression of photopic as well as sco-

TABLE D

COMPARISON OF ELECTRORETINOGRAM EVOKED BY WHITE PHOTOPIC AND RED PHOTOPIC STIMULI

Eye	White Photopic			Red Photopic		
	x-wave Amplitude (microvolts)	30/Sec. Flicker Amplitude (microvolts)	Flicker Fusion Frequency	x-wave Amplitude (microvolts)	30/Sec. Flicker Amplitude (microvolts)	Flicker Fusion Frequency
			I. CONTROLS			
A-11	150	175	75	125	*	70
A-15	125	125	60+	*	150	60+
A-17	175	155	55	110	88	55
A-19	100	100	60	75	60	45
A-20	150	125	65	125	125	60
A-21	150	175	65	150	100	60
A-22	175	125	70+	125	125	65
			II. EYES WITH MACULAR LESIONS			
C-14	150	175	75	125	100	65
C-15	175	175	75	125	100	65
C-8	150	155	55	90	75	50
C-16	125	150	±60	150	125	65
C-7	185	200	70	155	125	65
B-3	135	125	60	105	75	60
C-5	125	125	55	75	75	50
C-6	165	165	55	100	100	50
C-4	150	110	60	100	65	55
C-20	125	160	*	150	115	50
C-22	225	175	65	125	150	55

* Not measured.

topic potentials. Even assuming that at some stages of these retinal diseases one of the visual mechanisms is affected more than the other, and if sufficient studies were completed to establish statistically valid norms, it is doubtful that such small changes as might be found would be of real diagnostic importance.

The study of the photopic electroretinogram, however, is of importance where there is selective pathologic involvement of either the rod or cone mechanisms. Figure 2 shows the pattern of photopic-scotopic response in a normal subject, and in congenital night blind and totally color blind subjects. It is apparent that with the scotopic single flash (Karpe technique) alone, the congenital night blind would be erroneously diagnosed as having severe retinal pathology. This would be particularly misleading since the congenital night blind has normal visual acuity and color vision and generally functions well except at low levels of illumination.

In contrast, the color blind subject, who

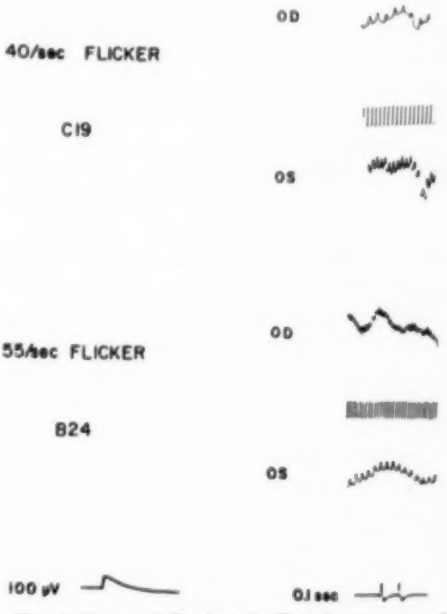

Fig. 3 (Iser and Goodman). The phenomenon of alternate response to flicker stimuli in eyes with retinal pathology as compared to the patient's other normal eye. (The identifying numbers refer to listing of pathologic eyes in Tables B and C.)

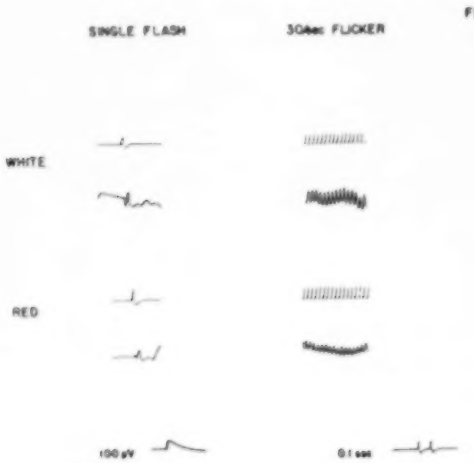

Fig. 4 (Iser and Goodman). The photopic electroretinogram in an eye with a solitary macular lesion, comparing the response to white and red stimuli.

is truly handicapped with photophobia and 20/200 vision, would have a normal electroretinogram as determined by the scotopic technique alone. The absence of the photopic potentials in this case demonstrates the cone deficiency, while their presence in the night blind subject is indicative of a functioning photopic visual system.

It must be concluded that for accurate evaluation of such subjects both photopic and scotopic electroretinogram studies must be performed and compared.

The correct diagnoses of retinal anomalies is of practical as well as physiologic importance. It is precisely in patients with a normal fundus and functional complaints that the electroretinogram is of major diagnostic value. The color blind subject in this study was considered to be a "learning problem" for many years and was referred for psychologic evaluation of a so-called functional complaint. Decreased vision from birth often leads to the interpretation of minor macular irregularities as "macular degeneration." The correct diagnoses may be made by electroretinography long before the child is able to co-operate for complicated psychophysical testing. The electroretinogram can also be of value in testing malingerers who feign the symptoms of total color blindness or night blindness.

An illustration of the diagnostic value of the photopic-scotopic electroretinogram is presented by the findings in three teen-age boys who were referred to the Ophthalmology Branch with similar complaints (fig. 5). Brief abstracts from their records follow:

Patient 1 (table C, No. 5 and No. 6) is a 19-year-old white male who presented with the chief complaint of defective vision since the age of eight years. On examination, visual acuity in the right eye was 20/100, and in the left eye 20/200. Small central scotomas in each eye were demonstrated by tangent screen examination. Funduscopic examination revealed loss of normal macular reflex and perimacular pigment changes. Electroretinogram was normal. This patient had been previously diagnosed as having optic atrophy and macular degeneration.

Patient 2 (table B, No. 18 and No. 19) is a 17-year-old white male who presented with the chief complaint of defective vision beginning at the age of six years and progressing until the age of 13 or 14 years. On examination, visual acuity was limited to finger counting at two feet in each eye. Funduscopic examination revealed both maculas to be slightly elevated with perimacular pigment disturbance. Color sense as measured by large colored objects was grossly intact. Adaptometry revealed an elevated rod

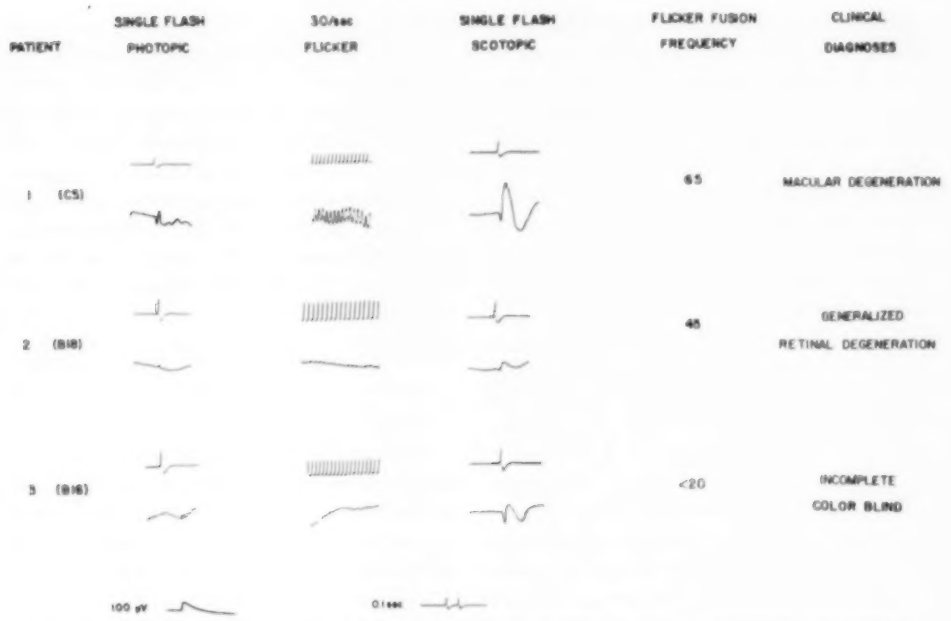

Fig. 5 (Iser and Goodman). The electroretinograms in three patients with decreased visual acuity. (The identifying numbers refer to Tables B and C.)

and cone threshold. The electroretinogram was generally depressed. Previous diagnoses included: (1) Leber's disease, (2) macular degeneration, (3) total color blindness.

Patient 3 (table B, No. 16 and No. 17) is an 18-year-old white male who presented with the chief complaint of defective vision since early childhood. On examination, visual acuity was 20/80 in each eye. Funduscopic examination was normal except for minimal perimacular pigment changes. Color testing with the HRR plates revealed a severe red-green defect. Adaptometry disclosed a high cone but normal rod threshold. The electroretinogram was similar to that of a completely color blind individual. A diagnosis of optic atrophy and macular degeneration had previously been made.

In the first two of these patients, fundus examination confirmed the diagnosis of macular degeneration. The electroretinogram, however, was normal in Patient 1, indicating a local lesion not involving the peripheral retina, while in Patient 2, with a depressed electroretinogram, a generalized depression of the neuroepithelium was evident.

Patient 3, on the other hand, with a questionable fundus lesion produced an electroretinogram similar to the total achromat. However, color testing indicated some residual function in the yellow-blue area. It is probable that this patient fits into a group of incomplete color blindness as described by Sloan,[11] with the cone function so depressed that it was not evident in the electroretinogram.*

A 10-year-old girl (L. M. H.) was referred to the National Institute of Neurological Diseases and Blindness because of the presence of congenital nystagmus. In addition she gave a history of defective vision, photophobia, and some difficulty with color vision. Her parents had previously been told that she had an "optic nerve lesion." On ex-

* Since the completion of this series, another previously undiagnosed totally color blind patient has been studied, again illustrating the diagnostic value of the electroretinogram.

amination, visual acuity was 20/200, O.U. There was a fine low amplitude pendular nystagmus more evident in the light. There was no evidence of optic atrophy, but there was some pigment disturbance around the macula. She was able to recognize only two of the figures on the HRR plates. The electroretinogram was totally lacking in photopic components and had a slightly decreased b-wave (225 microvolts). The electroretinographic results confirmed the clinical impression of total achromatopsia. This diagnosis was supported by psychophysical testing done by Dr. Louise Sloan, at the Wilmer Institute.

B. Macular lesions

The diagnosis of macular disease is important in assessing visual function in the presence of opacities of the ocular media and in the differential diagnosis of central visual loss. Henkes[12] has shown that cases of macular degeneration produce low scotopic potentials when they are secondary to generalized vascular disease, but isolated macular lesions do not affect the scotopic electroretinogram.[13, 14]

From Polyak's[15] studies it appears that the central foveal region, which makes up the retinal area generally involved in "macular" disease, contains only about 115,000 of the four to five million retinal cones. Since the electroretinogram is a mass response, this central area, comprising only a small fraction of the whole, could be expected to detract only a few microvolts from the total photopic potential, if it were totally destroyed. Current techniques of investigation are too crude to register changes of this magnitude, and even if instrumentation were available it is doubtful whether they could be differentiated from physiologic variations. This is borne out by the fact that in this study the photopic potentials are the same in normal eyes and eyes with macular lesions.

These patients were also tested with red light stimuli under conditions of light adaptation so as to provide the most favorable conditions for observing photopic response (fig. 4). It was apparent, however, that allowing for the decrease in intensity due to the filter there was no significant difference between the electroretinogram evoked by white photopic and red photopic stimulation.

It appears, then, that under the conditions of this investigation no more information in regard to lesions of the macula can be derived from the photopic than from the scotopic electroretinogram.

C. Evaluation of the various techniques of the photopic electroretinogram

As has been previously described,[16] the amplitude of the flicker response decreases at high frequencies until individual waves are no longer seen and stimulation produces no apparent change in the normal baseline activity. Variation in the "noise" level of the apparatus and artefacts produced by contraction of facial muscles and eye movement may materially affect the apparent fusion frequency. Furthermore as the fusion level is approached, responses may be observed to become irregular (fig. 3). All these factors make the choosing of the flicker fusion point a rather arbitrary matter.

Under circumstances where apparatus for producing flicker stimuli is not available, study of the x-wave itself may be an adequate measure of photopic response. Indeed, the single flash stimulus has an advantage in that it does not produce the photophobia and involuntary eye movements which may occur with high intensity flicker. However, in low amplitude records it is sometimes difficult to differentiate a single photopic response from a residual b-wave or recording artefact.

To overcome these factors the flicker waves at a frequency of 30/sec. have been chosen for study. Stimulating at this frequency assures photopic response free of scotopic potentials because the stimulation itself maintains a constant state of light adaptation. Fixation of this flickering light avoids disturbances caused by random movement

during recording, which may be a disturbing factor in single flash technique. At this frequency the response is a sinusoidal wave of maximum amplitude which is unaffected by baseline activity. Moreover, in this manner a large number of responses can be recorded in a very short time. Preliminary observations of the flicker wave at 30/sec. as reported in this study indicate that, in the majority of cases in which it was used, it paralleled the single flash photopic and flicker fusion results in a record which was much easier to analyze.

Summary and Conclusions

Twenty-five normal eyes and 50 eyes with ocular disease have been studied with single flash scotopic and photopic, and flicker photopic electroretinographic techniques.

The study of both the photopic and scotopic electroretinogram has its greatest diagnostic and prognostic value in conditions which selectively involve either the photopic or scotopic mechanism. It appears to be particularly valuable in the investigation of cases of congenital anomalies, as well as cases of hereditary degenerations.

In other types of retinal disease (for example, retinal detachment and chorioretinitis) where such selective involvement is not found, and in lesions involving the macula alone, the photopic recording was found to be no more informative than the scotopic. In addition, studies with red light stimuli show no significant variation from white stimuli in lesions of the central retinal area.

The retinal response to high intensity flicker light is a valuable method of recording photopic response and has some advantages over the single flash x-wave. However, it appears that the study of the amplitude of the flicker wave itself at a frequency of 30/sec. is more reliable than attempting to measure the point of fusion.

Photopic flicker recording is a useful adjunct to the standard scotopic single flash technique in clinical electroretinography.

National Institutes of Health (14).

Acknowledgment

Dr. Ralph Gunkel designed and constructed the plastic electrode holder used in this study (fig. 1).

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Discussion

Dr. Paul W. Miles (Saint Louis): I was most grateful for the opportunity to read this excellent paper before its delivery. It is a most comprehensive work. Dr. Iser and Dr. Goodman should be complimented for developing a new method of electroretinography. Flicker targets have been used before, but they were of the rotating sector disk type. In previous work, emphasis was placed on trains of waves produced by a continuously increasing or decreasing rate of flicker. Here, various frequencies of flicker target were used, but in distinct trains each of constant frequency. This permitted, at each frequency, an inspection of the a-wave and the x-wave which can only be seen at the onset of the response.

They developed the new method properly by physiologic studies on normal humans and on patients known to have a void of either rods or cones.

Their superior tracings may largely be due to their selection of the new Grass photostimulator. The importance of a brief intense flash compared to one which rises to a peak and gradually disappears in such minute time relations as an electrical response from the human retina should be obvious. With a brief intense flash, the a-wave is greater in amplitude and can better be distinguished from the slower rod reaction, the b-wave. The brief flash stimulates the retina to a strong but brief discharge, and does not leave it inhibited for a second flash for a long period of time.

This has been my explanation for the superiority of the brief intense flash for the testing of flicker fusion frequency. I have used this in daily office practice for several years and find it very useful. The electroretinogram with flicker may offer the advantage of an objective instead of a subjective test.

The chief objection I have found to flicker fusion fields is that in a series of normals there is as much as 15-percent rate variation. A given individual will repeat very well within about two percent. From the literature, it would seem that electroretinography has the same disadvantage. If the electroretinogram is as variable in normals as flicker fusion, perhaps the cause is retinal and not cerebral as I have believed. Do you have an explanation for this variation?

Iser and Goodman are also commended for using color stimuli with flicker electroretinography. Since either color or flicker will distinguish cone and rod response, their combination should be the ultimate. In your paper, you used red light at three to five flashes per second to produce excellent x-waves and also b-waves. With this in mind, how do you account for the results of Jacobson and Basar which were reported earlier this morning? They suggested a possible test for macular function in patients with cataract on the basis of electroretinograms from red light. In patients with known macular disease, red stimulation produced no response.

By this advanced method of diagnosis applied to some patients previously thought to have some form of optic atrophy or mental disease, Iser and Goodman have made a real contribution.

Dr. Hermann M. Burian (Iowa City, Iowa): I really should not be up here since I did not have the opportunity to study the papers prior to this meeting. These papers are so full of valuable and interesting information that one needs time to digest them before giving an intelligent discussion. I mainly want to thank the authors for their excellent work and for their presentation of it.

It seems to me that one point, brought out so well by these papers, is worth emphasizing and I do not get tired of reiterating it. This point is that electroretinography will not become a useful clinical tool until electroretinographic functional tests are developed. The use of colored filters, repetitive stimulation, graded intensities, and the like are all part of such functional tests.

There is, however, one word of warning which I should like to add. Neither the authors of the last paper nor Dr. Jacobson have mentioned the need of adjusting the intensity of the stimulus light when using, say, red filters. To my mind it is essential to do this. Unless the intensity is controlled, comparative studies with filters are not very revealing, since one can never be sure to what extent the effects obtained depend on the intensity of the stimulus rather than on the quality of the stimulating light.

Dr. Gilbert Iser (closing): There is no disagreement between our results and the results that Dr. Jacobson presented this morning. I did not have the opportunity of reading his paper in advance, but from what he said it appears that all or most of his patients were in the older age groups and had senile macular degeneration. This type of degeneration is usually accompanied by generalized retinal vascular disturbances and probably involves more of the retina than just the central macular area.

Our patients, for the most part, were in the younger age groups with solitary lesions caused mainly by chorioretinitis. In these cases the peripheral retina was normal. I think Dr. Jacobson is absolutely correct in his finding of a decreased photopic component in this group of patients in whom the macular degeneration was part of the generalized vascular disturbance. We have found the same thing. However, in patients with the solitary macular lesion in an otherwise healthy retina the photopic components are found to be normal.

We fully agree with Dr. Burian's comment as to the importance of regulating the intensities when comparing white with colored stimuli. As indicated in this presentation, we felt that the difference between the response to white photopic and red photopic stimuli was probably due to the intensity difference, rather than to any specific effect of wavelength.

THE EFFECT OF RATE OF RISE OF PHOTIC STIMULI ON THE HUMAN ELECTRORETINOGRAM

HANS BORNSCHEIN, M.D., AND RALPH D. GUNKEL, O.D.*
Bethesda, Maryland

Previous investigations of the stimulus-response relationships in the human electroretinogram have realt with the influence of stimulus factors such as luminance, area, duration, and spectral composition. No systematic study has been reported regarding the rate at which the light stimulus reaches its full intensity. However, there are a few observations suggesting a possible influence of this factor on the electroretinogram.

Early investigators (Kuehne and Steiner,[1] Ishihara[2]) could depress the retinal response of the frog by an extremely slow rise of the light stimulus. Bartley and Bishop[3] found that a "tapered" onset of the light stimulus in the rabbit produced the usual b-wave, though the latency of this wave was increased. Finally, Bornschein and Schubert[4] completely eliminated the a-wave of the human electroretinogram with slowly rising light stimuli, while little or no change was produced in the b-wave.

The present investigation deals with the effect of the rate of rise of the light stimulus on the human electretinogram, as compared with the effects produced by varying the stimulus intensity.

METHODS

The apparatus for producing stimuli of different rates of rise was constructed on the principle of the pendulum shutter. It consisted of a 15-watt fluorescent tube one inch in diameter and 13 inches long, with a luminance of about 3,000 millilamberts. The tube presented a rectangular test field subtending visual angles of about 60 degrees by five degrees. It was placed in the slit of a black surrounding field, which could be rotated through 90 degrees in a vertical plane before the subject's eye. The center of the tube was maintained at the same level as the eye, and approximately 11 inches in front of it.

A nine-foot pendulum, carrying a flat black rectangular shield, was suspended from the ceiling so that it could alternately occlude or expose the entire length of the fluorescent tube. To minimize eye movements, the subject was instructed to fix on a spot of light which was projected to a point on the pendulum shield corresponding to the center of the fluorescent tube. As the pendulum swung the spot remained stationary, so when the tube was exposed the subject's gaze was directed to the center of the tube.

In order to create the minimum rate of rise of the light stimulus, the fluorescent tube was held in a horizontal position and the pendulum was retracted to the place where its shield completely covered the tube. As the pendulum was released, increasing amounts of the tube were exposed until the entire length was uncovered in about 280 milliseconds. The pendulum was immediately returned to its position covering the tube. Duration of light stimulus was about one second.

The maximum rate of rise of the light stimulus was produced by turning the tube to the vertical position. As the pendulum was released, a displacement of one inch exposed the entire width of the tube, allowing a rise to full intensity in about 22 milliseconds. By rotating the tube to different angles between the horizontal and vertical positions, any rate of rise between the two extremes could be obtained.

To reduce the luminous flux incident upon the subject's eye, two different methods were employed. Varying proportions of the length or width of the tube were masked off by

* From the Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

means of linear diaphragms in order to reduce the stimulus area. With small stimulus areas, neutral filters (Inconel) were utilized to vary the luminance. Since area was varied as well as luminance, the term "stimulus intensity," as used in this paper, refers to the illuminance measured in foot candles at the subject's cornea. Although luminance and area are generally considered to be interchangeable factors in producing an electroretinogram of "standard" amplitude[5,6] differences in the intensity function of a-wave and b-wave have been described as a result of an inverse variation of luminance and area.[7] Therefore, the fact should be stressed that in the present study, where comparisons were made on the effect of rate of rise at a given intensity, the relation between luminance and area was kept constant for every variation of rising time.

Radical reduction of stimulus intensity without reducing area was obtained by means of a red filter (Wratten No. 29). This was particularly effective because the b-wave is scotopic in nature and has a low sensitivity to red light. The red filter also absorbs the wave lengths which predominate in the spectrum of the fluorescent tube, leaving a relatively low total transmission. There is no doubt that the slow positive wave produced by red stimuli is a true b-wave, as demonstrated in another study.[8]

The electroretinogram was recorded in the usual way, using a contact lens electrode (Burian-Allen). The amplifier system consisted of a capacity-coupled pre-amplifier (Grass P-5) which was connected with one of the direct-coupled main amplifiers in a double-beam cathode ray oscilloscope (ETC K26). Photographic records were taken by means of a kymograph camera (Grass C-4-D). The over-all time constant of the system was 0.9 seconds (lower limit of frequency range less than one per second).

Preliminary studies were done on six normal subjects. With the stimulation technique used in the present investigation, it was difficult to avoid disturbing eye or lid movements which occurred during recording of delayed and prolonged responses. Therefore, most of the records were taken from two subjects (C. B., D. H.) selected because of stability of baseline.

The electroretinographic recordings were begun after 30 minutes of dark adaptation. The light stimuli were separated by two minute intervals to minimize light adaptation. The pupils were immobilized by complete dilatation (10-percent neosynephrine).

The material evaluated in this study consists of 391 records.

Results

Figure 1 shows the changes in the amplitude of the a-wave and b-wave produced by altering rate of rise or intensity of light stimulus. Examples of the original records are included in Figure 2. Prolongation of rising time from 25 to 100 milliseconds reduces the a-wave to about half its amplitude, while the b-wave amplitude remains almost constant. With a further increase of rising time to 230 milliseconds the a-wave is

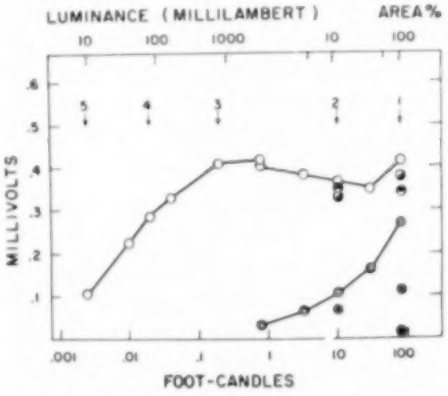

Fig. 1 (Bornschein and Gunkel). Subject C. B. Amplitude of the negative (◎ ⊙ ●) and the positive (○ ◕ ◓) response in the human electroretinogram with variation of intensity and rising time (white light stimuli). Intensity = illuminance at the cornea (ft-c) varied by luminance (mL) and area (percent of maximum value = 13 sq. in.). Rising times: 25 milliseconds (◎ ○), 100 milliseconds (⊙ ◕), and 230 milliseconds (● ◓). Every point represents the mean value of five determinations.

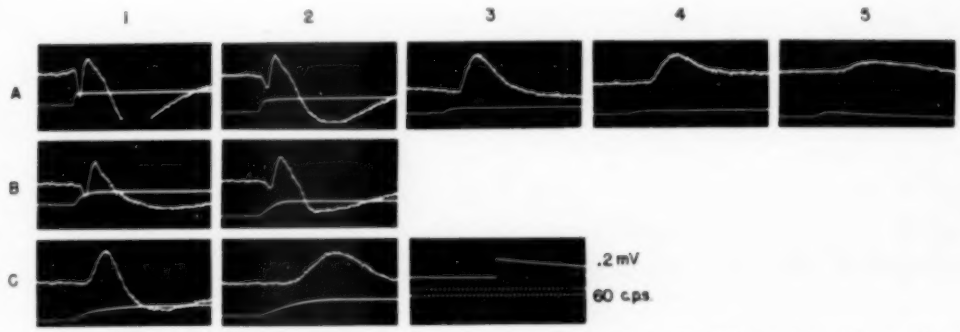

Fig. 2 (Bornschein and Gunkel). Subject C. B. Electroretinogram with different intensities and/or rising times of white light stimuli. The numbers (1-5) refer to the intensities marked in Figure 1 by arrows and corresponding numbers. Rising times: A = 25 milliseconds, B = 100 milliseconds, C = 230 milliseconds.

usually extinguished, whereas the effect on the b-wave is essentially a lengthening of the response.

The changes in the electroretinogram caused by decreasing rate of rise are somewhat similar to the effects produced by reducing stimulus intensity (figs. 1 and 2). This raises the question whether rate of rise and intensity can be considered as interchangeable stimulus factors. With supramaximal stimuli the amplitude of the b-wave is little influenced by changes in stimulus intensity. Therefore, it was necessary to study this question, using stimuli which elicit a submaximal response, where there is an approximately linear correlation between intensity and amplitude (fig. 1). For technical reasons noted previously, red light stimuli were used which produced a b-wave of about half the maximum amplitude. Figure 3 shows the electroretinogram in response to three such stimuli, equal in intensity but differing in rate of rise. With the longer rising times the b-wave appears markedly prolonged, without any change in amplitude. Therefore, the variations effected in the electroretinogram by modifying the rate of rise of the stimulus are of a different nature than those produced by alteration of intensity.

No conclusion can be drawn from the present study with respect to the photopic x-wave. The second increase of the positive response, which normally occurs with high intensity stimulation, is presumably caused by a superposition of b-wave and x-wave;[9] with long rising times this second "spurt" is missing (figs. 1 and 4). This could suggest

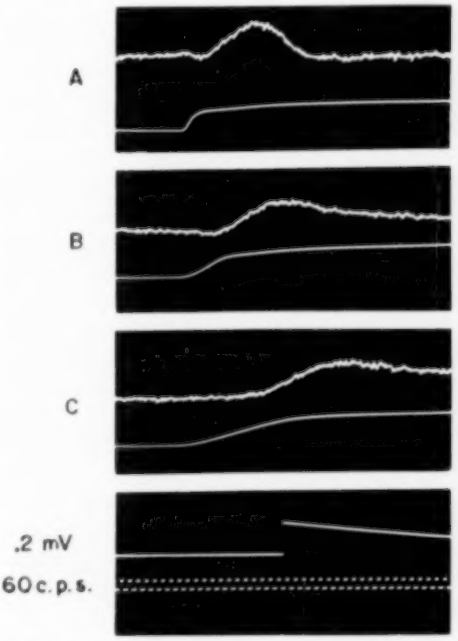

Fig. 3 (Bornschein and Gunkel). Subject C. B. Electroretinogram with different rising times of a low intensity stimulus (red light, Wratten 29). Rising times: A = 40 milliseconds, B = 100 milliseconds, C = 280 milliseconds.

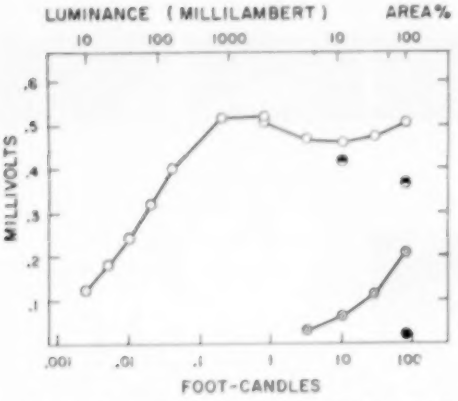

Fig. 4 (Bornschein and Gunkel). Subject D. H. Amplitude of the negative (◎ ●) and the positive (○ ◓) response in the electroretinogram with variation of intensity and rising time of light stimuli (white). Rising times: 22 milliseconds (◎ ○), and 260 milliseconds (● ◓). Every point represents three or four determinations.

that the x-wave is depressed in a manner similar to the a-wave. Unfortunately, under the present experimental conditions, no complete separation of the x-wave from the b-wave could be attained with red light stimuli. Further studies are necessary to obtain direct information about the behavior of this wave.

Recently, Johnson and Bartlett[10] have described a prolongation of the positive response in the human electroretinogram as an effect of increasing the duration of the stimulus. Therefore, it seems of interest that similar variations in the temporal aspects of the response can be produced by two different time factors of the light stimulus—rate of rise and duration.

The effect of rising time on the b-wave may possibly be related to the fact that the human electroretinogram represents a response to changes in light, as well as to light itself. If the temporal gradient of increasing illumination is extended over a longer period of time, the process underlying the b-wave may also continue longer. Whether there is a critical rising time, corresponding to a minimal stimulus gradient, cannot be answered on the basis of the present results.

Though the different behavior of the a-wave and the b-wave must be noted as fact, any theoretical interpretation would be purely speculative. However, it seems of some practical interest with respect to the use of rotating disc shutters in flicker electroretinography. If the stimulating light beam is cut outside its focus, every decrease in the flicker frequency by diminishing the speed of the disc increases the rising time of the light stimuli, resulting in a selective reduction of the a-wave at low frequencies.[4]

The simple method of stimulation, used in the present study to vary intensity and rate of rise is necessarily limited in its application. An improved technique should allow the production of longer rising times, which would necessitate the use of a direct coupled amplifier system for measuring protracted responses. Accordingly, the present study can only be considered as a pilot experiment showing the importance of a hitherto neglected stimulus factor in the human electroretinogram.

Summary

This study deals with the changes in the human electroretinogram produced by varying the rate at which the light stimulus reaches its full intensity. With slowly rising stimuli the a-wave can be eliminated, whereas the characteristic change of the b-wave is a prolongation of the response. The results are discussed with regard to the influence of other stimulus factors on the electroretinogram.

National Institutes of Health (14).

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Discussion

Dr. Werner K. Noell (Buffalo, New York): This paper demonstrates a phenomenon of which most electroretinographers have been well aware; but nobody to my knowledge has taken the pains previously really to quantitate the relationship between the rising time of the stimulus and the electrical response.

The rising time of the stimulus is, of course, of very great importance as are all the other parameters of stimulation. Unless these parameters are defined and measured, electroretinographic data at best only apply to the laboratory in which they are obtained. I am afraid that the many efforts which have been made in obtaining "standards" for the normal electroretinogram may in the long run prove wasted because the stimulus machine which was used has either limited applicability or cannot truly be rebuilt by other laboratories. Especially in view of Dr. Bornschein's paper I am tempted to say that we should achieve first a standardization of the stimulus properties and then establish what is a "normal" response in terms of amplitudes, latent times, threshold, and so forth. Of course, every laboratory can determine its own "normals" but this is obviously not the best procedure.

With respect to the specific results of this study may I direct your attention to the fact that the electroretinogram—as recorded clinically in man—is the sum of two potentials of opposite polarity. These two potentials, as we all know, have a different latent period of appearance, a different time course, and also a different stimulus-response relationship as first shown by Granit. In order to record the a-wave at all an instantaneously strong stimulus must be applied. But—and this is the point I would like to make—the absence of the a-wave with lower stimulus intensities does not indicate that these stimuli do not produce a cornea-negative component at all. What changes with lower stimulus intensity is the rising rate of the a-wave process and its "latency" in relation to that of the b-wave. In our animal experiments, all low intensities which provoke a measurable b-wave produce a cornea-potential negative when the b-wave is selectively abolished by the use of certain poisons. Moreover, it became obvious to us recently that the latent period of these cornea-negative potentials (in the absence of the b-wave) can be shorter than the latency of the low-stimulus electroretinogram under normal conditions. Hence the "normal" low-stimulus electroretinogram may be preceded by a short time period during which the early fraction of a- and b-potentials cancel each other. This would imply that the full time interval between stimulus and electroretinogram is not a "latent period" in the strict sense of this term. Perhaps this also could provide an explanation why a slow rising time of the stimulus abolishes the a-wave as shown today in this very fine paper.

STUDIES ON THE VISUAL TOXICITY OF METHANOL*

IX. The effect of ethanol on methanol poisoning in the rhesus monkey

Anita Peek Gilger, M.D., Albert M. Potts, M.D., and Irene S. Farkas, M.S.
Cleveland, Ohio

That small, repeated doses of ethanol would have a salutary effect upon the course of methanol poisoning was first suggested in 1904 by an anonymous Army surgeon whose opinions were published by Wood and Buller.[1] These authors state: "It is the belief of Assistant Surgeon R., from his experience of five cases of poisoning, that methyl must be replaced by ethyl alcohol, in order to combat a collapse and sustain the patient's vitality."

Wood and Buller reported the five cases of poisoning treated by Lieut. R., but dosages of ethanol are not clear. The only man to survive without blindness apparently received 30 cc. of whiskey every hour for about a day. The other survivor, who became blind, received an initial dose of "whiskey 8 cc. hypodermatically;" whiskey was continued but dosage was not stated. Presumably the three fatal cases were also given whiskey. In addition to ethanol, treatment in these five patients consisted of varying amounts of strychnine, potassium iodide, enemas, heat, and galvanic electricity. Wood and Buller apparently agreed with Lieut. R. because they advocated that therapy consist, among other medications, of "stimulants, especially ethyl alcohol, strychnia and coffee."

Røe, starting in 1943,[2,3] reawakened interest in ethanol therapy of methanol poisoning. He stated that the consumption of ethyl alcohol was the chief cause of the great tolerance toward methanol shown by some individuals, and that ethyl alcohol could prolong the latent period and reduce severe symptoms of poisoning by preventing acidosis. Røe's views were based on clinical observations of cases singled out from his larger series; however, statistical analysis would be subject to the notorious lack of reliability about the amounts of alcohols imbibed by human subjects. From the experimental work on alcohol metabolism available in the literature at that time, Røe concluded that the mechanism of action was "the capacity ethyl alcohol possesses to displace methyl alcohol from the inner surfaces of cells, its oxidation to formic acid being thereby checked." He suggested that ethanol be used in conjunction with alkali therapy or alone when intravenous bicarbonate could not be given. He recommended that, using gin containing 45 vol. percent ethanol, a man be given 133 ml. as his first dose and 20 ml. every hour afterward.

In addition to Røe's cases ethanol in conjunction with alkali has been used therapeutically in human cases of methanol poisoning by Chew, et al.[4] and by Agner, et al.[5] Røe has published[6,7] comments on these cases. However, dissident opinions have been voiced by other clinicians (Bennett, et al.[8]).

Experimentally in man, Zatman[9] and Leaf and Zatman[10] found in three human subjects that, when ethanol was ingested simultaneously with methanol, the concentration of methanol in the urine was much increased. Zatman[9] and Kendal and Ramanathan[11] using alcohol dehydrogenase also reported in vitro experiments in which they found that the dehydrogenase was able to oxidize methanol at only about one-ninth of the rate for ethanol and that there was a competitive inhibition of methanol oxidation when both methanol and ethanol were present simultaneously, inhibition being demonstrable

* From the Laboratory for Research in Ophthalmology, Western Reserve University, and the Ophthalmology Service, Department of Surgery, University Hospitals of Cleveland. This work was aided by grants from the U. S. Public Health Service, National Institute of Neurological Diseases and Blindness, and the Ohio Lions Club.

when the molar ratio of ethanol to methanol was only about 1:16. Zatman concluded that, if the theories that the toxic effects of methanol are due to its oxidation products be correct, then "the experimental results described above would lead one to expect ethanol to diminish the toxicity of methanol, permitting the excretion in an unchanged condition of a larger fraction of the ingested dose."

Kendal and Ramanathan,[12] using two of the same human subjects previously used by Leaf and Zatman, first gave methanol alone and later gave ethanol simultaneously with, and every hour for five hours after, the same amount of methanol. They confirmed the previous findings of increased urinary methanol when ethanol was given and also found that the appearance of formate was completely inhibited by the administration of ethanol but reappeared one to two hours after the administration of ethanol ceased. They concluded "that a primary oxidation of methanol to formaldehyde through the agency of alcohol dehydrogenase is competitively inhibited by ethanol."

All other experimental works on the effect of ethanol on methanol poisoning in vivo have used nonprimate test objects. Previous work from our laboratory[13] indicated that only in other primates is there close similarity to human methanol poisoning. For this reason we shall not review the literature dealing with nonprimates. However, in view of the findings to be reported, we call attention to our previous report[14] that ethanol increased the toxicity of methanol in white mice and our recommendation at that time that "use of ethyl alcohol in the therapy of human methanol poisoning should be discontinued pending further investigation." In a later publication[15] based on the study of methanol poisoning in the rhesus monkey we described the threefold primate disease—only the first phase of which, the alcohol narcosis, was seen by us in subprimates. We are in agreement with Røe[7] that the increased ethanol toxicity in mice was due to the additive narcosis of ethanol plus methanol. Thus these findings appear to have no bearing on methanol and ethanol effects in the lower doses significant for primates.

Obviously when man is used as a test object, lethal doses of methanol cannot be given. Hence we continued our study of the effect of ethanol on methanol poisoning using the rhesus monkey as the experimental animal. The following is the report of this work.

Material and Methods

Five young male rhesus macaques were used. Methanol was made up in 20 percent (by weight) solutions and ethanol in 10 percent (by weight) solutions. In some of the experiments five-percent sucrose or glucose was added to the solutions. See Table 2 for details.

The monkeys were given all solutions by stomach tube. Single, presumably lethal, doses of methanol were given at the start of the experiment together with small, repeated doses of ethanol. In the event of survival the monkeys were given three weeks (except monkey 4 who had two weeks) to recuperate and then were given the same dose of methanol alone.

The choice of methanol dosage was based upon our laboratory's previous experience with untreated methanol poisoning in rhesus monkeys, which is listed in Table 1. The minimum lethal dose apparently lay between 2.0 and 3.0 gm./kg., and we expected that 6.0 gm./kg. would be the approximate LD_{100}. The first monkey was given 4.0 gm.

TABLE 1

Mortality in the Rhesus macaque from single oral doses of methanol.*

Dose g MeOH/kg body wt.	Total Number of Monkeys	Number Surviving	Number Dying
2.0	3	3	0
3.0	3	1	2
4.0	1	0	1
6.0	1	0	1
8.0	1	0	1

* 20% (by weight) solution.

TABLE 2

CLINICAL EFFECTS OF ETHANOL ON ACUTE METHANOL POISONING IN THE RHESUS MACAQUE

	Monkey	Date of Experiment	Dose 20% MeOH (gm./kg.)	MeOH Lost by Vomiting (gm./kg.)	Dose 10% EtOH (mg./kg.)	Other Fluids	Eye Examinations*	Clinical Results
1.	Elpenor wt. 2.0 kg.	7-12-55	4.0	0	0.67 initially 0.40 q.4 h.x 9	0	Normal	Survived
	wt. 2.8 kg.	9-12-55	5.0	0	0.67 initially 0.40 q.4 h.x 9	0	Normal	Survived
	wt. 2.9 kg.	10- 1-55	5.0	0	0	0	Normal	Died at 26 hr.
2.	Erebus wt. 3.0 kg.	11-20-55	6.0	0	0.75 initially 0.50 q.4 h.x 16	0	Normal	Survived
	wt. 2.9 kg.	12-12-55	6.0	0.15 at 3 hr.	0	0	Normal	Died 18–19 hr.
3.	Elagabalus wt. 2.6 kg.	1-29-56	6.0 5% sucrose added	0.2 at 4 hr.	0.75 initially 0.50 q.4 h.x 15 5% sucrose added	0	Normal	Survived
	wt. 2.3 kg.	2-19-56	5.8 5% sucrose added	0	0	Total 270 cc. 5% sucrose during 48 hr.	From days 2 to 4 after MeOH right pupil larger than left and R.E. had hyperemia of disc and retinal edema.	Survived — Remained sick and died 18 da. after MeOH.
4.	Epandamanus wt. 4.9 kg.	3-19-56	6.0 (58%)	0	0.75 initially 0.50 q.4 h.x 14 5 g. glucose added with doses 2 to 6	0	Normal	Survived
	wt. 4.2 kg.	4- 2-56	6.2	0	0	0 Ate and drank normally until 31 hours after MeOH.	O.U.: Retinal edema; hyperemia and later blurring of margins of discs.	Died 36–38 hr.
5.	Esau wt. 3.9 kg.	4-15-56	6.0	0	0.75 initially 0.50 q.4 h.x 15	0	Normal	Survived
	wt. 3.2 kg.	5- 6-56	6.0	0.05 at 2 hr.	0	Total 105 cc. 5% sucrose	Normal	Died 18–19 hr.

* Pupillary changes associated with coma are not reported.

methanol/kg. with ethanol. In the second and third experiments this monkey was given 5.0 gm. methanol/kg. The other four monkeys were given 6.0 gm. methanol/kg.; minor losses due to vomiting are recorded in Table 2.

Choice of ethanol dosage was initially based on that suggested by Røe.[3] We assumed that Røe was referring to a 70-kg. man; thus his dosages would be 0.67 gm./kg. initially and 0.10 gm./kg. every hour thereafter. The shortest practical interval for us to use between doses was four hours. Therefore we gave 0.40 gm./kg. every four hours for 36 hours after the initial higher dose. The laboratory data resulting from experiments with the first monkey were such that we slightly increased the ethanol dosage for the other four monkeys to: 0.75 gm./kg. initially with the methanol. 0.50 gm./kg. every four hours for 56 to 64 hours.

Since the ethanol therapy added sizeable fluid volumes and some calories, compensation was made as follows in the untreated experiments (exact details given in table 2):

1. Two monkeys had volumes of 5-percent sucrose equivalent to the volumes of ethanol used in the preceding experiments, but given usually at longer intervals than every four hours.

2. Three monkeys received no additional fluids. One of these three was conscious, and eating and drinking until 31 hours after the methanol, with death occurring before 37 hours. The other two monkeys died by 19 and 26 hours so that dehydration could not have been a factor in their deaths. Sucrose

or glucose were irregularly added (for details, see table 2) for the initial purpose of minimizing vomiting. This was not convincingly efficacious. Sucrose was added to the final untreated experiment (Monkey 5) purely as a gesture toward control. In view of the variability of the monkey diets prior to, and in some cases during, the experiments, the addition of these sugars should have no significance.

The animals were closely watched for the first six hours after methanol administration so that any vomitus during this period could be collected separately and analyzed for methanol content. Bloods were collected before poisoning with methanol and at suitable intervals thereafter, as is shown in the tables. They were usually drawn just prior to the administration of ethanol. External and ophthalmoscopic examination of the eyes were made at the same times. Plasmas were frozen until analyses were made. Carbon dioxide combining capacity was determined as described previously.[16]

Results

A. Clinical

Table 2 summarizes the doses of methanol and ethanol given and our clinical results in the five rhesus monkeys. For comparison with human doses for a 150-pound man, 6.0 gm./kg. methanol equals 1.4 fifths of 50 vols. percent methanol, and 0.75 and 0.50 gm./kg. ethanol equals 4.5 and 3.0 oz. of U.S.P. (100-proof) whiskey. (See discussion.)

When treated with small repeated doses of ethanol, all five of our monkeys survived a large, single dose of methanol. When given the same dose of methanol alone, four out of the five died. One monkey of the five survived 6.0 gm./kg. of methanol without ethanol treatment. His course thereafter was steadily down-hill. He died 18 days after being given methanol alone so that unfortunately we could not learn whether some systematic error was responsible for the anomaly or whether ethanol would protect him against a larger dose of methanol. Sections of his brain showed no pathologic changes attributable to methanol poisoning.[17]

It is of interest, however, that this sole surviving monkey was one of the two monkeys in untreated experiments to show eye changes typical of human poisoning. Since eye damage usually presents itself late, it is not surprising that in the untreated experiments the three other monkeys, who died early following massive doses of methanol, did not show eye damage. Most important, none of the monkeys showed any eye damage during or after the treated experiments.

B. Plasma carbon-dioxide combining capacity

Severe acidosis is a frequent finding and a cause of death in primate methanol poisoning. Charts 1 and 2 show the CO_2 combining capacities for two typical monkeys; acidosis data from all experiments are reported in Table 3. Chart 1 (Monkey 1) shows the acidosis which occurred after premature cessation of ethanol. For 36 hours, during which he had received treatment, the monkey maintained CO_2 combining capacities ranging between 31 and 49 vol. percent. At the last ethanol dose he had 41 vol. percent; only seven hours later he had 22 vol. percent. This comparatively moderate acidosis persisted until the fourth day after poisoning. Because of the rapid onset of acidosis in this experiment, we not only slightly increased the ethanol dosage in subsequent experiments, but also prolonged the period over which it was given to at least two and a half days after poisoning. This first chart also shows the severe acidosis produced prior to death by the same amount of methanol untreated.

The second chart (Monkey 2) shows that with the increased and prolonged ethanol treatment no acidosis occurred, although this monkey was given a larger amount of methanol than the first one. Actually the lowest CO_2 combining power in the experiment was that of the normal plasma. This chart shows again the development of a

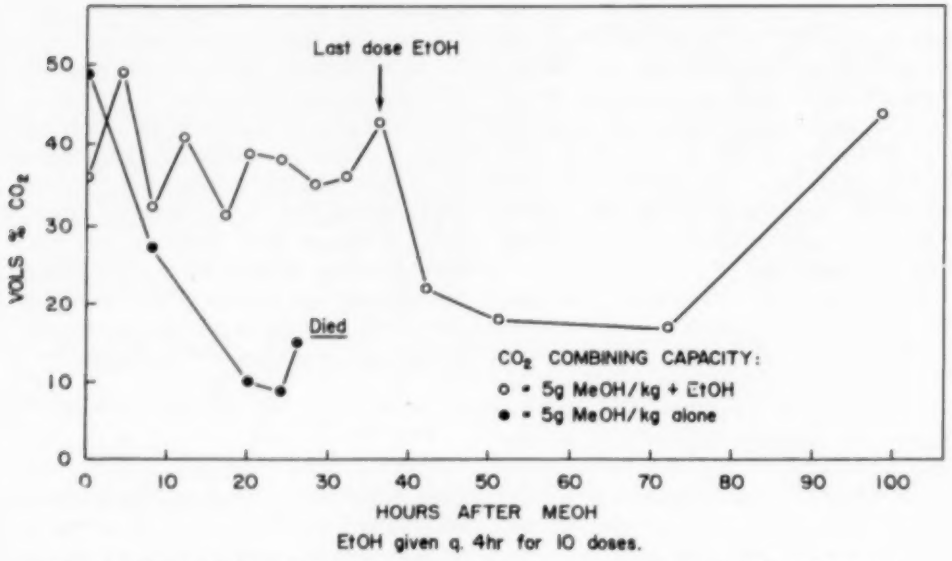

Chart 1 (Gilger, Potts, and Farkas). Effect of ethanol on acute methanol toxicity in Monkey 1.

severe acidosis prior to death when the same animal received the same dose of methanol untreated.

Table 3 shows that none of the other three monkeys developed severe acidosis when treated with ethanol. The two who died when untreated both became several acidotic prior to death. The one monkey to survive the methanol untreated did not become acidotic.

Discussion

It is evident from the foregoing results that ethanol is an effective agent against methanol poisoning in the rhesus monkey. Because of the apparently unique nature of the disease in primates these results are in agreement with those of clinical investigators who have used ethanol therapy on large numbers of cases; and we add our recommenda-

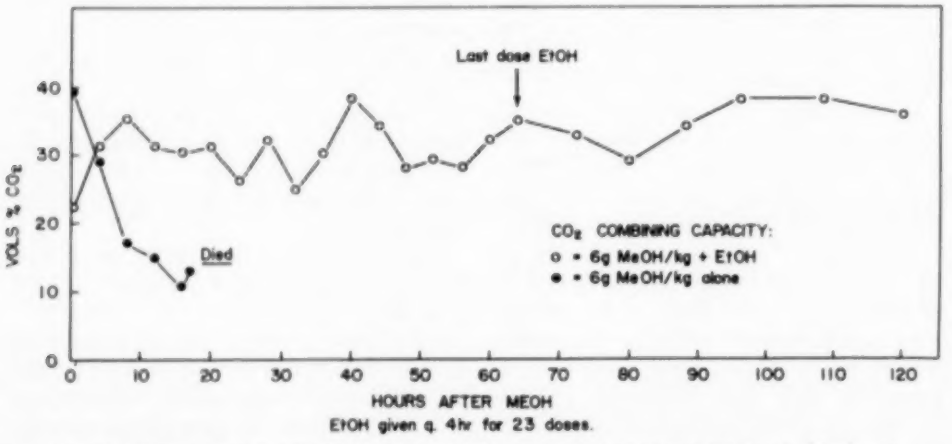

Chart 2 (Gilger, Potts, and Farkas). Effect of ethanol on acute methanol toxicity in Monkey 2.

TABLE 3

EFFECT OF ETHANOL ON ACIDOSIS CAUSED BY ACUTE METHANOL POISONING IN THE RHESUS MONKEY

Plasma CO_2 Combining Capacity (Vols. %)

Time After MeOH (Hours)	1. Elpenor			2. Erebus		3. Elagabalus		4. Epandamanus		5. Esau	
	4 gm. MeOH and EtOH	5 gm. MeOH and EtOH	5 gm. MeOH Alone	MeOH and EtOH	MeOH Alone	MeOH and EtOH	MeOH Alone	MeOH and EtOH	MeOH Alone	MeOH and EtOH	MeOH Alone
0	42.1	36.1	49.0	22.3	38.5	38.7	54.0	37.6	30.6	23.3	24.4
4	41.6	49.1		30.9	28.7		62.7	50.8			
8	37.6	32.3	27.3	34.8	16.9	50.8		48.5		43.0	29.9
12	41.9	40.6		31.3	15.6			53.3			
16	45.4			29.7	10.6	30.2		46.4		38.9	20.7
17		31.0			12.5						
20	35.2	38.8	9.5	31.2	(died)			32.8	23.5		10.5*
24	45.4	38.0	8.9	26.1		38.5	47.8	34.0	21.2	33.5	(died)
26			14.8*								
27			(died)						22.7		
28	40.9	35.0		31.8			50.3	57.4			
32		35.7		26.4		36.5	34.4	48.9	14.7	43.6	
35	19.3										
36	47.8	43.2		29.5				40.6	17.8		
40				38.1		44.2	29.6	46.2	(died)	45.6	
42		22.1									
44				33.8				30.8			
48				27.9		39.4	35.6	34.8		44.6	
51		17.9									
52				29.2				30.2			
56				27.9			42.7			45.7	
60				31.8		35.8					
64				34.8							
72		16.9		33.1		31.9				44.0	
76							44.7	21.6			
80				28.7							
85						38.7					
86				34.1							
96		43.8				48.8					
Day 5											
A.M.				37.7		45.6	58.2			43.4	
P.M.				37.7							
Day 6				35.2		49.6	43.0			37.0	
Day 14								30.6			
Day 18							50.1*				
							(died)				
Day 19		49.0									
Day 21						54.0				24.4	
Day 22				38.5							

* Post-mortem heart puncture.

tions to those previously published, notably by Rǿe, that ethanol in small, frequently repeated doses be used to treat human methanol poisoning.

The implications of these experiments for therapy are best understood if one converts ethanol and methanol values to familiar terms. A dose of 6.0 gm./kg. of methanol would be equivalent in a 70-kg. man to ingestion of 1.4 fifths of 50 vol. percent methyl alcohol. The treatment with ethanol would correspond to ingestion of 4.5 oz. of U.S.P. whiskey (100 proof) followed every four hours by 3.0 oz.

The results also throw more light on the mechanism of methanol poisoning in the primate and subprimate as well. As mentioned previously by us,[15] methanol poisoning in the primate is a threefold disease:

Disease 1 is due to the narcotic action which methanol shares with the other aliphatic alcohols and, for that matter, with the other organic solvents. This is due to methanol itself and is manifested in all animals at dosage levels in the neighborhood of 10 gm./kg. This is the only one of the three diseases seen in lower animals. Since it is a nonspecific organic solvent narcosis, it is evident that the increased deaths with ethanol seen in our mice[13] were due to the summation of this organic solvent effect at the 10 gm./kg. dosage level. It is just conceivable that, if the initial dose of methanol were sufficiently high in the human, addition of

ethanol therapy could similarly result in increased fatalities (see calculations above for relative doses). This should at least be borne in mind.

Disease II is seen in primates only, and at lower doses of methanol than 10 gm./kg. It is characterized by a latent period before it begins to be manifested and is invariably connected with an uncompensated metabolic acidosis. In the light of the work of Zatman,[9] Leaf and Zatman,[10] and Kendal and Ramanathan[11, 12] in vitro and in vivo and of the results of ethanol treatment on humans and monkeys, it seems reasonable to conclude that Disease II is caused indirectly by an oxidation product of methanol. When ethanol is present in even modest concentrations it successfully competes with methanol for alcohol oxidase and is preferentially oxidized to innocuous products. If this situation can be maintained for a sufficiently long time, the methanol will be excreted as such (urine, expired air), no methanol oxidation products will be formed, and Disease II will never appear. Evidence from this laboratory[10] indicates that formic acid is not found in sufficient concentration to account for the acidosis found in Disease II, and it is likely that the symptoms result indirectly from the creation of some more toxic substance, presumably formaldehyde.

If Disease II is combatted by base, the subject survives for a much longer period; and Disease III becomes manifest. In untreated animals and patients where the methanol dose is not high enough to cause death in acidosis before 30 hours, some manifestations of this disease can also be seen. Disease III is characterized by eye effects and central nervous system lesions—retinal edema, fixed dilated pupil, blindness, and basal ganglion necrosis—as described previously.[13] When ethanol was given with methanol to our monkeys, Disease III also did not appear. It is logical to assume that Disease III is an additional result of poisoning by the oxidation product of methanol and that treatment of the animal with base allows time for this third manifestation to appear.

A number of questions arise in regard to ethanol therapy, and some of these are answerable by further experiments on monkeys. The first of these is the question of how long after a specified dose of methanol will ethanol therapy be effective. If Diseases II and III are due to a metabolite of methanol which is being formed during the typical latent period, one would expect to find ethanol therapy ineffective after the initial onset of acidosis and probably for some time before this. Experiments are in progress to determine what are the actual parameters involved.

The question of against how large a dose of methanol one may protect is also of interest. On the basis of the theoretical considerations mentioned above, one would expect a significant blood level of ethanol to protect against any dose of methanol, providing it could be maintained until the methanol was completely excreted, and providing both alcohols did not reach the 10 gm./kg. range. This aspect, too, will be explored further.

In the absence of any final answers to the above questions one must still outline a tentative procedure for treatment of human cases. A suggested procedure is the following: Any individual suspected of receiving a toxic dose of methanol should be hospitalized and blood should be drawn for immediate determination of CO_2 capacity, methanol, and ethanol. If no signs other than inebriation are present, the patient should be started on ethanol therapy, scheduled as outlined earlier in this section. That perhaps this dosage schedule may not be quite adequate for humans is suggested by some of our recent clinical experience. In several instances the blood ethanol level fell to negligible values toward the end of the four hour period. Thus more frequent or somewhat higher doses may be indicated. In our present state of knowledge, ethanol therapy as well as alkali therapy should require the continuing treatment to maintain adequate blood levels, the close observation,

and the frequent analytical checks afforded by hospitalization. Many victims of methanol poisoning in the literature have been found on the second day to be normal and have been sent home, only to return moribund after a sudden collapse. If successful, this treatment with ethanol should prevent the onset of acidosis, eye signs, or other methanol effects.

If signs of acidosis are present, base should be given in addition until the CO_2 combining capacity has returned to normal. Here again it is impossible to give specific amounts and dosage schedules for oral bicarbonate or intravenous lactate, but these must be adjusted to the severity of acidosis. Iatrogenic alkalosis and overhydration are to be avoided equally with underdosage. Because of the rapid onset of acidosis and its persistence, signs of its occurrence must be watched for continuously and treatment must be prolonged until acid production has ceased. On the basis of our experimental work and the human cases reported by Benton and Calhoun[18] (see discussion by Potts), there is no evidence that administration of base combats anything but Disease II. When acidosis has set in, however, administration of base is a necessary and often lifesaving measure.

Thus, to date, we have in ethanol a prophylactic agent against Diseases II and III and in base a direct therapy for Disease II. The problem of combatting the proximal toxic agent responsible for Diseases II and III and the problem of direct therapy for Disease III remain to be solved.

Summary

1. Five rhesus monkeys, who were orally administered single, large doses of methanol, survived when treated with small, repeated doses of ethanol. When given the same dose of methanol alone, four out of the five died. One monkey of the five survived a large dose of methanol without ethanol treatment; he died 18 days after he had been given methanol.

2. All five monkeys showed no objective signs of eye damage following methanol poisoning when treated with ethanol.

3. Ethanol therapy started simultaneously with methanol poisoning prevented occurrence of the typical severe acidosis.

4. Theoretical implications of this study were discussed. We agree with Røe's recommendation that ethanol therapy be used in human methanol poisoning.

University Hospitals of Cleveland, 2065 Adelbert Road (6).

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Discussion

Dr. Walter H. Fink (Minneapolis): I wish to congratulate Dr. Gilger and her associates on their valuable contribution to this complex problem.

Because of the research on this subject carried out in the laboratory, and on clinical cases, we have reached a therapy in methyl alcohol poisoning where there is a fair assurance not only of saving life, but also of preventing serious complications.

Therapy has two main objectives, first, to neutralize the acidosis, and, second, to prevent the development of acidosis.

It was recognized after the work of Harrop and Benedict in 1920 that methyl alcohol produces acidosis in human beings, but the importance of alkalinization was not appreciated until 1946 when the reports of Chew and of Røe made it evident that severe manifestations could be influenced by prompt and adequate use of sodium bicarbonate. It is now recognized that combatting the intense acidosis is the most important feature of therapy. Treatment of the acidosis with sodium bicarbonate has been found to be highly effective when adequate doses are given and at a sufficiently early stage of the poisoning. It is most important to bear in mind that the risk of recurrence of the acidosis is very great and necessitates that the treatment be continued over a period of time. All other therapeutic measures now available must as yet be considered secondary to the main objective of prompt restoration of plasma bicarbonate.

The second objective in treatment of methyl alcohol is the prevention of acidosis.

It has been demonstrated that the oxidation of methyl alcohol is inhibited by ethyl alcohol. An explanation is that some of the methanol becomes absorbed on the constituents of the cells. When ethyl alcohol is given, methanol is displaced from these areas and the concentration of methyl alcohol in the fluid of the organism becomes higher than it was before. Ethyl alcohol must consequently be assumed to inhibit the oxidation of methanol and the production of formic and other acids will cease.

Further evidence of the inhibiting effect of ethyl alcohol is suggested by the fact that patients who had taken ethyl alcohol, as well as methanol, suffer less from the poisoning than those who had taken methanol only.

Despite the experimental basis for its therapeutic use, clinical confirmation of ethanol's efficacy is not yet established. In the beginning much contradictory literature concerning the therapeutic value of ethyl alcohol came from laboratory experiments and confused the issue. As is now well known, laboratory animals, such as dogs and rabbits, do not respond to the effect of methyl alcohol as do the humans. It seems, however, that the animals used in this project would respond more like the human and therefore the findings are more significant.

The clinical reports to date concerning the use of ethyl alcohol are not conclusive in most instances because alkali was used in conjunction with the ethyl alcohol. To cite a few examples, Chew administered whiskey to 26 patients in his series and all recovered but all were also treated with large doses of alkali. There was no correlation of ethanol consumption and time of onset of symptoms, their severity, or the ultimate results. In the series reported by Keeney and Mellinkoff, blood ethanol levels in the fatal cases were usually much higher than blood methanol.

Agner, Hook, and Von Porat have reported the treatment of two human cases of methyl alcohol poisoning treated with ethanol and alkali, but the results are difficult to assess, especially in view of the fact that one of the patients died. Branch and Tonning administered ethanol to one patient with inconclusive results (alkali was also given).

Because it is rarely possible to start treatment early enough in humans to get the inhibiting effect of the ethyl alcohol, the use of ethyl alcohol is therefore looked upon by many as a supplementary measure after the acidosis has been treated with sodium bicarbonate. They believe that the recurrence of acidosis will be prevented if, after the first

treatment of bicarbonate, ethyl alcohol is given and continued by giving small doses equivalent to the quantity undergoing oxidation.

Others believe that it is not necessary to give ethyl alcohol when we are able to give bicarbonate under constant control of the alkali reserve or the reaction of the urine. But when it is not feasible to give bicarbonate, as for example during a prolonged transport to the hospital, Røe recommends the use of ethyl alcohol.

Others believe that ethyl alcohol is contraindicated in view of its depressant action and the fact that these patients may also be severely intoxicated with ethyl alcohol at the outset.

Consensus at present seems to indicate that the role of ethyl alcohol in the treatment of methyl alcohol poisoning in the human should be supplementary to the alkanization but should not substitute for it.

Dr. Gilger's work is a valuable contribution to our knowledge of this difficult subject and has given us answers to some of the questions related to the obscure data. I hope she will continue this work and eventually, if the opportunity arises, confirm her laboratory findings on the human.

Dr. Albert M. Potts (closing): There is just one point that we wish to emphasize, and that is that we see no prospect in the immediate or any other future of being able to carry out such experiments on humans.

The results with human treatment, with the urgency for saving life, have caused this confusion between the effects of base and the effects of ethanol. As far as any human case reports have shown, there has been no effect of alkali treatment in preventing either the visual effects or the effects on the central nervous system. Whereas akali must be used as a lifesaving measure, the thing that will eventually save vision, if one can extrapolate from our primate experimental animals, is something which prevents the oxidation of methanol in the first place.

The acidosis could obviously be treated by base. However, the acidosis itself is probably not a direct effect of oxidation of methanol but a secondary effect, as we have pointed out in the past. There is not enough formic acid excreted; or in fact, if all the methanol were converted to formic acid, there still would not be enough acid to cause acidosis.

Therefore, we believe that the acidosis itself is a secondary result of damage by the same metabolic product which causes Disease III (that is, eye damage). So there is a very good reason, in the light of these experiments, for using ethanol even though base is being used.

ROD AND CONE VISION IN RETINITIS PIGMENTOSA*

Bernard H. Zeavin,† M.D., and George Wald,‡ Ph.D.
Cambridge, Massachusetts

Although the subject of continuing research, retinitis pigmentosa remains largely mysterious. The etiology still eludes us, and no treatment is available. A strongly hereditary character, slow progressive course, the lack of a therapeutic agent, and high incidence combine to make retinitis pigmentosa one of the most serious of ophthalmic problems.

The clinical features are well known (Bell, 1922; Fralick, 1950; Carroll, 1950). The two main symptoms are an early history of night blindness followed by a progressive loss of peripheral fields leading eventually to so-called gun-barrel vision and its attendant visual incapacity. The ophthalmoscopic picture of bone corpuscle-shaped deposits of black pigment in the retina is characteristic. On occasion, no pigment deposits are found (so-called retinitis pigmentosa sine pigmento). There may also be some narrowing in the caliber of the retinal arterioles. Both eyes are usually involved equally.

At present, there are two main methods of following the course of the disease. The less reliable is to observe changes in the appearance of the fundus. A slightly better gauge of the disease's progress is obtained by checking by perimetry the narrowing of visual fields. The first method is wholly qualitative; the second yields only an arbitrary outer boundary of the residual functional retina with no information regarding the

* From the Biological Laboratories, Harvard University, Cambridge, and the Howe Laboratory of Ophthalmology, Boston.

† Hovey Fellow in Ophthalmic Research. Present address, U. S. Army Hospital, Fort Dix, New Jersey.

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state of affairs inside the boundary. On the whole, little is known regarding which of the visual cells, rods or cones, are attacked first or the further course of the degeneration. Cogan (1950) found that the rod and cone layer had entirely disappeared in all his cases except occasionally for a few abnormal cones in the central area and in the far periphery.

Several attempts have been made in recent years to analyze more closely the loss of visual capacities in retinitis pigmentosa. Mandelbaum (1941) showed that dark adaptation exhibits characteristic changes in this disease. In a paracentral region of the retina, the thresholds of both rods and cones rise, but rods much more rapidly than cones, so that the interval between the two narrows progressively with the course of the disease. Cone thresholds are abnormally high in all but the mildest cases, in the fovea as well as in the retinal periphery. Furthermore, measurements of the dark adapted threshold across a retinal meridian ("threshold contours") display a striking inversion when compared with normal: in the normal retina the threshold is maximal at the fovea; in retinitis pigmentosa it is usually minimal (see also Sloan, 1939, p. 244). These observations have been confirmed by Haig and Saltzman (1947), who have demonstrated also a progressive loss of central visual acuity in retinitis pigmentosa (1955).

The present investigation attempted, through measurements of visual threshold and adaptation, to establish quantitatively the functional status of rod and cone vision in the surviving retina. A special perimetric adaptometer was designed, and a procedure employed which singles out the thresholds of rod and cone vision in any desired area of retina within 40 degrees from the fixation point. A series of traverses across various meridians of the retina yield what are effectively functional cross-sections ("profiles") of rod and cone vision within the areas of measurement. Such profiles specify accurately the retinal condition at the time of measurement, characterize the effects of the disease, and may in future provide an accurate index of its course and of the effects of therapeutic agents upon it.

I. A PERIMETRIC ADAPTOMETER

This instrument, shown in Figures 1 and 2, consisted of a test unit and a brightness control panel. The control panel was approximately the same as described by Wald

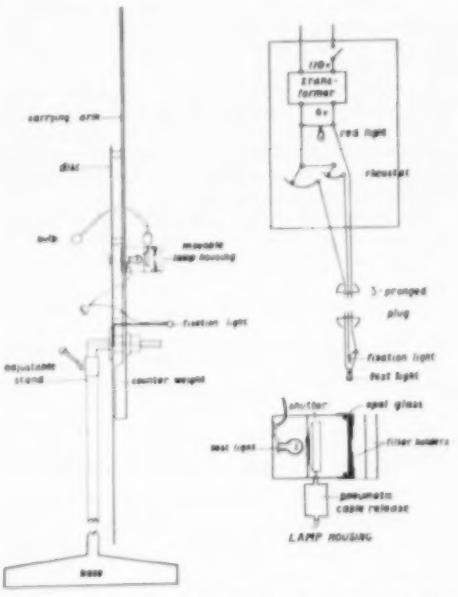

Fig. 1 (Zeavin and Wald). Diagram of the perimetric adaptometer. (Left) An adjustable stand holds a large, revolvable aluminum disc, slotted to coincide with a similar slot in the carrying arm, through which pass the hose from the pneumatic cable release and wires from the test and fixation lamps. The fixation lamp is held just far enough in front of the disc to allow the lamp housing to be brought directly behind for making foveal measurements. A counterweight attached to the carrying arm balances the weight of the lamp housing. (Upper right) Simplified diagram of the electric circuit. The part enclosed in the control box is drawn within the rectangle and up to the three-pronged plug, while the part joined to the test unit is on the other side of the plug. After passing through a six-volt transformer and supplying a red light by which the operator works, it leads to two rheostats in series with the fixation and test lights, providing for separated control of their brightnesses. (Lower right) Enlarged sagittal section of the lamp housing.

Fig. 2 (Zeavin and Wald). The perimetric adaptometer in use. The subject is seated with her head in an adjustable chin rest 81 cm. in front of the test unit. At this distance, the test field subtends an angle of one degree with the eye. The operator is shielded by the black disc so that the small red light by which he works does not disturb the subject. The pneumatic cable release of the shutter is operated with one hand, while the brightness control dial is regulated with the other. All tests were monocular, one eye being covered with a patch.

in his portable visual adaptometer (1941). It was connected by a long cord to the test unit. It contained two rheostats for separate control of the fixation light and the test light. The rheostat for the test light had a regulating dial calibrated for brightness. There was also a transformer to reduce 115 v. to 6 v.

The test unit consisted of a wide revolving disc with a slot carrying the housing of the test lamp. This provided a means for varying the position of the test light in relation to the fixation light, which was stationary and placed near the center of the disc. The lamp housing was adapted from Wald's portable adaptometer (1941). It contained a lamp with a shutter, an opal glass, and two filter holders. It was shorter and hence lighter than the housing previously described, and, since the lamp was closer to the opal glass, provided much higher brightnesses.

Test unit

A side view of this is shown in Figure 1, along with a sagittal section of the lamp housing. The lamp housing had a removable rear cap which bore a radio pilot light receptacle with a six- to eight-volt, 0.15-ampere lamp. Since this was almost always used at lower than the rated voltage it lasts almost indefinitely. Immediately in front of the lamp was a camera shutter, to which was attached a pneumatic cable release. Just in front of the shutter was the opal glass, before which was placed a metal diaphragm with a circular opening 1.4 mm. in diameter. The image of this subtends on the retina a visual angle of one degree when the subject's eye is 32 in. (81 cm.) from the instrument. The front cap contained channels for holding two-in. square neutral or color filters.

The revolving disc was mounted on a stand adjustable for height. The disc, 32 in. in diameter and made of aluminum, had a slot coinciding with a similar slot in the carrying arm. This allowed the hose of the pneumatic cable release and wires from the test and fixation lamps to pass back to the operator from the lamp housing. The wires from the lamps to the control panel were interrupted by a three-pronged plug, so that the panel could be separated from the test unit.

The fixation point was provided by a miniature lamp held in a metal clamp about five in. in front of the disc, allowing just enough room for the lamp housing to be brought directly behind it for making foveal measurements. A pyrex rod, painted black except for its tip, extended out from the lamp so that its tip fell exactly in the center of the test light when in place for foveal measurements. The tip alone showed as a bright point of light, the fixation point.

The carrying arm was 33-in. long and had a slot in it 25-in. long. At one end was

attached a counterweight to balance the weight of the lamp housing and its accessories. The slot carried the lamp housing out to 40 degrees from the fixation light, under the conditions of our experiments. The lamp housing could be placed in any quadrant by revolving the disc, and could be moved in and out along the carrying arm by rack and pinion. The operator never had to emerge from behind the disc except for changing filters.

On the back of the disc, both sides of the slot were marked in degrees, indicating the distance of the test light from the fixation point. There was also a small protractor indicating the angle at which the carrying arm projected. The entire instrument was painted a dull black.

Brightness control panel

Figure 1 shows also a simplified diagram of the electric circuit. The part enclosed in the control box is drawn within the rectangle and up to the three-pronged plug, while that of the circuit joined to the test unit is on the other side of the plug. Within the control box, the circuit first passed through a six-volt constant voltage transformer and, having provided for a small red light by which the operator worked, led to two rheostats in series with the fixation and test lights. These gave independent control over the brightness of the two lights, a necessary arrangement for measurements on patients with retinitis pigmentosa, since the fixation light at times needs to be much brighter for them than for normal subjects.

Calibration

To have the lamp housing of the test light small enough to be moved along the carrying arm, we had to devise a small and light means of regulating the intensity of the light. We resorted to the "remote" control of a rheostat. This, however, has the consequence that the spectral distribution (color temperature) of the lamp changes with the resistance thrown into the circuit, and hence with the brightness. Both spectral distribution and brightness independently influence visual threshold. This disability was circumverted by calibrating the whole range of brightnesses employed with a Macbeth illuminometer. In this way, we measured brightness for the human eye, regardless of wavelength composition, in terms of the equivalent standard white light of this instrument.

It turned out that log brightness was linear with the scale readings on the rheostat. The useful range of the instrument was about 2.3 log units, but this range was extended by inserting neutral filters.

The two color filters selected for our work were Wratten 22 (orange) and Corning 4305 (blue). The intensity range was calibrated through both filters and exhibited with both a linear relation between scale readings and log brightness.

Procedure

The adaptometer is shown in operation in Figure 2. The subject was seated with his head in an adjustable chin rest 81 cm. in front of the test unit. At this distance, the test field subtends an angle of one degree with the eye. The shutter was set at 1/25 second. The operator stood behind the test unit, shielded by the revolving disc so that the small red light by which he worked would not disturb the subject. With one hand the operator clicked the shutter, while with the other he turned the dial of the panel, controlling brightness.

All tests were monocular, one eye being covered with a patch. During a measurement, the subject was instructed to fixate steadily. Starting at a subthreshold intensity, the subject was exposed to flashes of gradually increasing brightness until he reported just seeing one of them. In circumstances in which the threshold was not changing, readings were repeated at least twice. Between readings, the subject closed his eyes.

II. Measurements with the normal eye

Rods and cones

In the passage from dim to bright light, the spectral sensitivity of the eye shifts toward the red. This is the familiar Purkinje phenomenon, and is caused by differences in the spectral sensitivities of rods and cones. Figure 3 shows the average spectral sensitivity curves of 22 normal subjects, measured separately for rod and cone vision, eight degrees above the fovea (Wald, 1945; 1949). Our procedure for identifying rod and cone vision can be understood best with reference to this diagram. It is apparent that any two colored lights, however chosen, are effective in some constant ratio for stimulating cones, and in some other fixed ratio for stimulating rods (Wald, 1941).

In most cases we measured thresholds alternately through the Wratten 22 filter, whose dominant wavelength is about 580 mμ (orange), and the Corning 4305 filter, whose dominant wavelength is about 520 mμ (blue). It can be seen in Figure 3 that in rod vision the threshold measured through the blue filter lies 0.90 log units *lower* than the orange; whereas in cone vision the "blue threshold" lies 0.16 log units *higher* than the orange. These ratios therefore specify whether a threshold involves rod or cone vision and can be used to analyze the participation of rods and cones in vision.

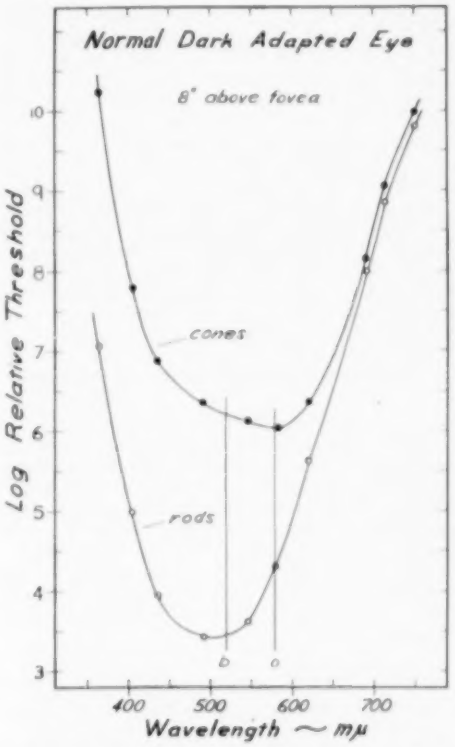

Fig. 3 (Zeavin and Wald). Spectral sensitivity curves of normal observers, measured eight degrees above the fovea. The rod measurements are the averaged data of 22 observers: the cone measurements are averaged from 10 of the same observers (Wald, 1945). The two vertical lines are the dominant wavelengths of the color filters used: *o* = orange Wratten 22 filter with dominant wavelength at 580 mμ; *b* = blue Corning 4305 filter with dominant wavelength at 520 mμ. On the rod curve, the threshold for the *b* filter lies below that for the *o* filter; on the cone curve this relation is reversed.

Dark adaptation

With the perimetric adaptometer, dark adaptation can be measured in any region of the retina within 40 degrees of the fovea. The subject was first light adapted by five minutes' exposure to a brightness of 2,000 foot-candles. This was turned off, and the first measurement of threshold made immediately. Thereafter, thresholds were measured at intervals of about one minute for about 45 minutes. When the logarithm of the visual threshold is plotted against time in the dark, the familiar dark adaptation curves result. In an area containing both rods and cones, these show an initial steep fall of threshold to a first plateau, the dark adaptation of the cones; followed by a second descent of threshold to a second and final plateau, the dark adaptation of the rods. In the fovea, which contains only cones, only the first segment is observed.

Figure 4 shows a pair of dark adaptation

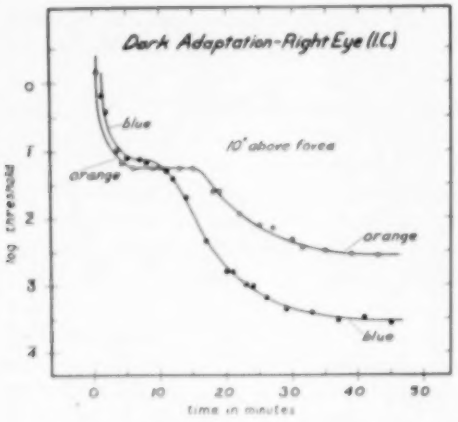

Fig. 4 (Zeavin and Wald). Dark-adaptation curves measured simultaneously 10 degrees above the fovea, in either orange (Wratten 22) or blue light (Corning 4305). In the initial period of dark adaptation, the orange threshold lies *below* the blue (cone thresholds), whereas later the orange threshold lies *above* the blue (rod thresholds). In the transition stage, both types of receptor co-operate in the thresholds, and the ratio for the two colors is intermediate.

curves measured simultaneously 10 degrees above the fovea,* in orange and blue light. The most interesting feature of these curves is the relationship of the blue and orange thresholds as the adaptation progresses from cones to rods. In the beginning, when cones are involved, the orange threshold lies about 0.14 log units below the blue; whereas later, as rods take over, the blue threshold lies about 0.95 log units below the orange. In the transition stage in which both types of receptor co-operate in the thresholds, the ratio of thresholds for the two colors varies between these extremes.

Retinal profiles

For these the subject was dark adapted for 30 minutes. Then visual thresholds were measured along the vertical and horizontal meridians of the retina passing through the fovea.

The visual thresholds are low in the peripheral retina, where rods are present, and rise to a peak at the fovea, where only cones are involved. They lie approximately at the same level in all quadrants except inferiorly, where they are generally higher.

Figure 5 shows retinal profiles of the fully dark adapted normal eye in the vertical and horizontal meridians. In the periphery, one observes the rod ratio of thresholds to blue and orange light, while at the fovea the reversed ratio characteristic of cones is obtained. Around the edge of the fovea is a transition zone in which both types of receptor participate in the threshold.

Figure 6 shows the retinal profile of another fully dark adapted eye, passing through the fovea in the vertical meridian. The same figure also shows the retinal profile of the dark adapted cones in the same meridian. The cones dark adapt in about four minutes, and from four to about nine minutes, when the rod thresholds intervene, one can measure the cone plateau. Throughout this interval, the cone ratio of blue and orange thresh-

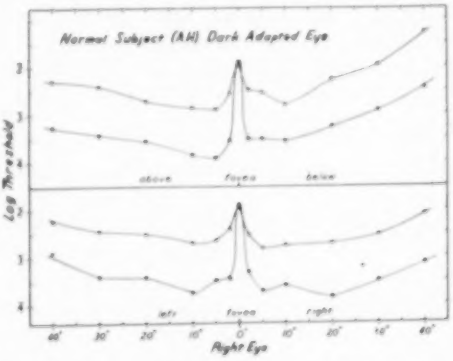

Fig. 5 (Zeavin and Wald). Retinal profiles of the fully dark-adapted normal eye in the vertical and horizontal meridians. Open circles represent thresholds to orange light, closed circles thresholds to blue light. In the periphery, the rod ratio of thresholds prevails, while at the fovea the reversed ratio characteristic of cones is obtained. Around the edge of the fovea is a transition zone in which both types of receptor participate in the threshold.

* In all cases the location of the field is stated in terms of the position of its image on the retina, that is, in the present instance the one-degree field was centered 10 degrees below the fixation point, so that its image on the retina fell 10 degrees above the fovea.

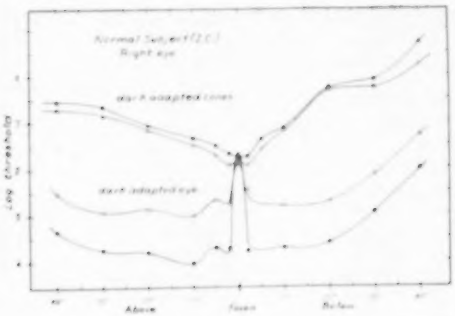

Fig. 6 (Zeavin and Wald). Retinal profile of another fully dark-adapted normal eye in the vertical meridian, along with the retinal profile of the dark-adapted cones in the same meridian. Open circles and triangles represent thresholds to orange light, and closed circles and triangles thresholds to blue light. The cone thresholds are highest in the extreme periphery, dropping gradually to a minimum about two degrees parafoveally, then rising slightly again at the fovea.

olds is observed. By taking measurements during this period, we record the visual thresholds of completely dark adapted peripheral cones. The profile of such measurements across the retina shows that the cone thresholds are highest in the extreme periphery of the retina, dropping gradually to a minimum about two degrees parafoveally, then rising slightly again at the fovea.

III. Retinitis pigmentosa

The types of measurement described were applied to the study of retinitis pigmentosa. We will begin by describing measurements on several subjects exhibiting early stages of the disease in which considerable peripheral vision remained; and go on to advanced cases, and to one case exhibiting special features.

Case 1

J. T. C. was 21 years of age with 20/30 vision in each eye individually, 20/20 binocularly. His fundi showed the typical bone corpuscle-shaped pigment deposits over most of the periphery except superiorly. The retinal arterioles were small but not thread-like. His peripheral fields (fig. 7) were relatively extended inferiorly, but limited in all other areas to about 15 to 20 degrees from the fixation point, except nasally in the left eye where the field extended out to about 45 degrees. The central fields (fig. 7) showed a ring scotoma in both eyes, broken only in the inferior meridian in the right eye, and broken in the left eye in the inferior and temporal meridians. Both fields were measured with a three-mm. white test object.

The horizontal and vertical retinal profiles of both eyes are shown in Figure 8. Compared with normal profiles, which peak at the fovea, the general configuration of the curves was strikingly inverted, the fovea retaining its normal thresholds while those of the periphery had risen about four log units, to levels much higher than the fovea. The foveal thresholds therefore form a minimum instead of the normal maximum. This inverted type of retinal profile was characteristic of all the retinitis pigmentosa patients we studied. The great rise in peripheral thresholds explains the symptom of nightblindness.

The comparison of thresholds in orange and blue light revealed that the thresholds of the completely dark adapted eye retain the characteristics of cone vision to two degrees parafoveally, and even to five degrees in the inferior meridian. In these regions, rod vision had either disappeared or become less

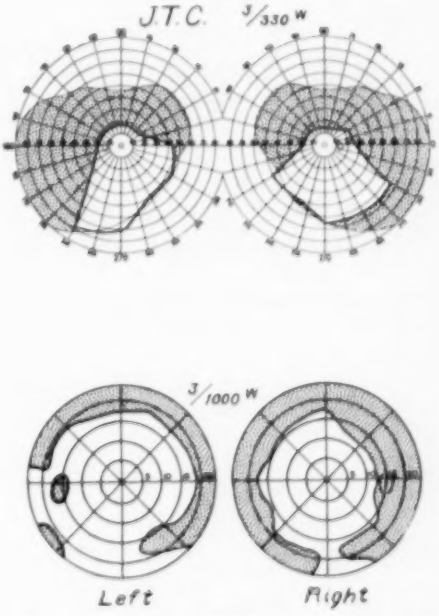

Fig. 7 (Zeavin and Wald). Case 1. (Top) Peripheral fields, relatively extensive below, but restricted in all other areas. (Bottom) Central fields show a ring scotoma in both eyes, broken only in the inferior meridian in the right eye, and in the inferior and temporal meridians in the left eye. Both fields measured with a three-mm. white test object.

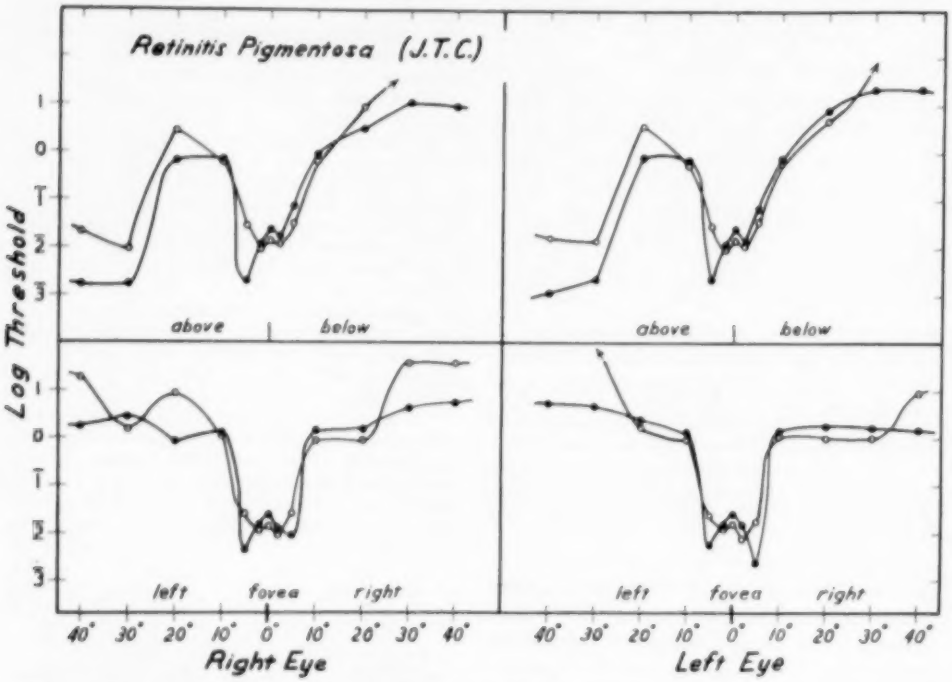

Fig. 8 (Zeavin and Wald). Case 1. Horizontal and vertical retinal profiles of both eyes. Compared with normal profiles, which peak at the fovea, the general configuration of the curves is strikingly inverted, the fovea retaining its normal threshold while thresholds in the periphery have risen about four log units to a level much higher than the fovea. Open circles represent orange light, closed circles blue light. The spacing of thresholds in orange and blue light reveals that many areas of the periphery retain the characteristics of cone vision, where normally rod vision prevails.

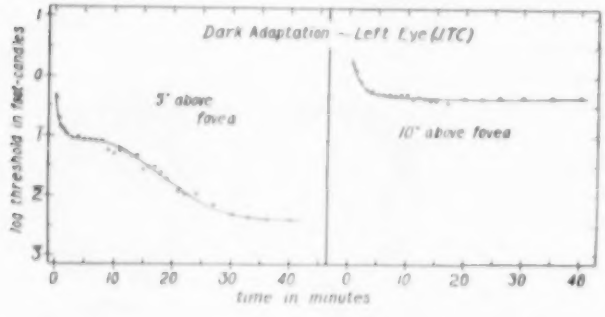

Fig. 9 (Zeavin and Wald). Case 1. Dark-adaptation curves measured with a white test light. Five degrees above the fovea, the curve is duplex, showing that both rods and cones are present. The normal threshold level of the cone plateau implies a normal complement of cones, but the high rod plateau indicates damage to rod function. Ten degrees above the fovea the curve is simple and typical of cones; rod function is apparently absent. The cone threshold is higher than normal, indicating damage to the cones also in this region.

sensitive to light than cone vision, which apparently remained relatively undisturbed. At five degrees parafoveally, except in the inferior meridian, rod vision was still present, but with a greatly elevated threshold.

Such increases in threshold could be caused by decreases in the number of functional receptors, in their individual sensitivities, in their capacities to summate, or any combination of these changes (Wald, 1938). Dark adaptation curves measured five degrees above the fovea (fig. 9) revealed the presence of both rods and cones. The normal threshold level of the cone plateau implies a normal complement of cones, while the high final threshold of the rod curve indicates that the rod apparatus had begun to fail.

Ten degrees from the fovea, in all four meridians, thresholds to orange and blue light characteristic of cone vision were again observed, but much higher than normal. Here, rod vision had either disappeared or become less sensitive than cone vision, which was itself somewhat impaired. Dark adaptation curves 10 degrees above the fovea (fig. 9) confirmed this condition. In further support, spectral sensitivity curves were measured in both eyes five degrees and 10 degrees above the foveas (fig. 10). Typical rod curves were obtained five degrees above the foveas, and typical cone curves 10 degrees above. Both rod and cone thresholds, however, were displaced about two log units above normal levels, again indicating functional damage.

Examination of the retinal profiles (fig. 8) revealed that none of the peripheral retina was normal. In most places only cone vision could be measured, usually impaired. Nearest to normal were the peripheral areas 30 degrees and 40 degrees above the foveas. Yet, even here, rod vision was damaged. On the other hand, at no place within the 40 degree radius was there a complete absence of both rod and cone vision, that is, total blindness. The retinal profiles corresponded very well with the examination of the fundi and the visual fields. In general, however, the profiles tended to reveal a larger area of functional retina than the examination of visual field.

CASE 2.

F. J. C. was the 24-year-old brother of J. T. C. (Case 1). Although he had just been discharged from the Army because of retinitis pigmentosa, we had no report of the examination of his fundi and fields. His retinal profiles (fig. 11) were measured for comparison with those of his younger brother (fig. 8).

The characteristic inverted shape of the retinal profile was found once again. The fovea was normal, as in Case 1. Also similar to Case 1 was the abnormality of the entire peripheral retina, again with some retention of retinal function in all areas examined. Although rod thresholds were observed in most meridians five degrees parafoveally, the disease seemed to have damaged the retina somewhat more than in the same areas of his younger brother, since the thresholds were all higher. The distribution of the lesions in the two brothers was remarkably alike, with the disease perhaps a little more advanced in the older one (case 2).

CASE 3.

J. E. C. was the 18-year-old sister of the patients in Cases 1 and 2, and the only other sibling. She had no ocular complaints, and had never had her eyes examined. No information was available, therefore, about the appearance of her fundi or the extent of her fields. Her retina profiles (fig. 12) were measured for comparison with those of her brothers (figs. 8 and 11).

The inverted shape of the retinal profiles, characteristic of retinitis pigmentosa, was immediately evident. The foveas once again were the only normal parts of the retinas. Five degrees parafoveally, rod vision was observed everywhere, but obviously damaged. However, the damage seemed less severe in these areas than in her brothers. In addition, some rod vision could be measured in many places two degrees parafoveally, whereas in her brothers rod vision could not be measured in these areas. It appears therefore that though there had been no clinical history, the disease clearly was present in this girl, although less severe than in her brothers.

In this family, apparently the father and the paternal grandmother had the disease, but neither was examined by us. The fact that all the siblings were afflicted with the disease suggests a dominant hereditary

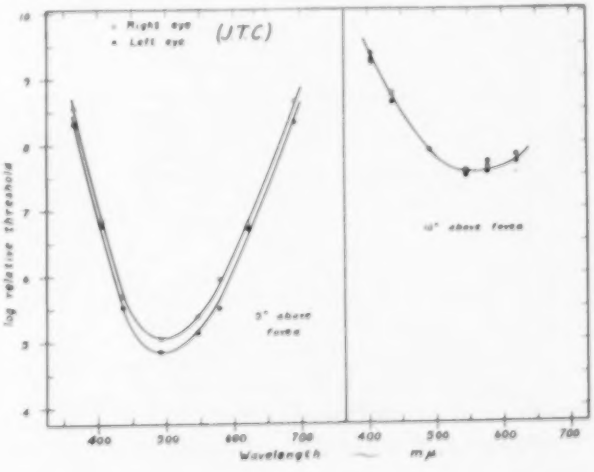

Fig. 10 (Zeavin and Wald). Case 1. Spectral sensitivity curves measured in the completely dark-adapted eyes, five and 10 degrees above the foveas. Five degrees above the foveas, typical rod curves are obtained, 10 degrees above the foveas typical cone curves. In both cases the curves are displaced about two log units above normal levels, indicating retinal deterioration.

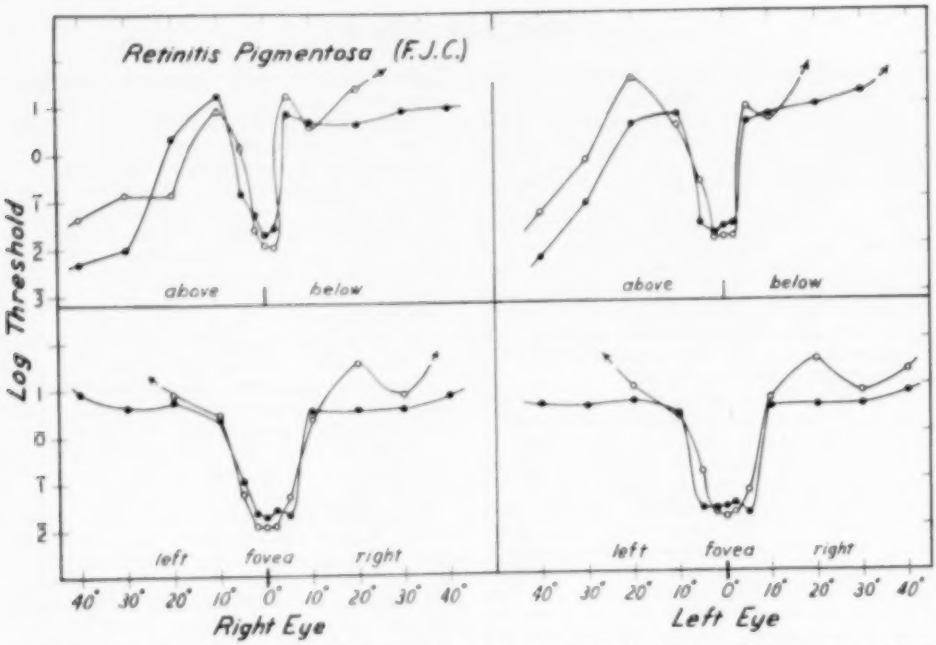

Fig. 11 (Zeavin and Wald). Case 2. Retinal profiles in the horizontal and vertical meridians of both eyes. Open circles represent orange light and closed circles blue light. The inverted configurations of all the profiles are characteristic of retinitis pigmentosa.

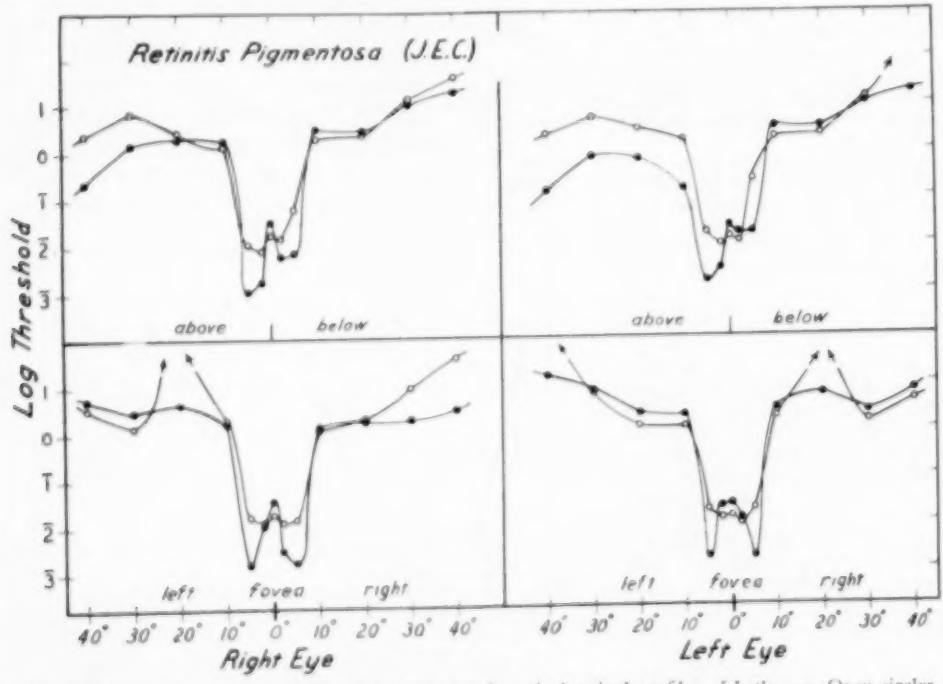

Fig. 12 (Zeavin and Wald). Case 3. Horizontal and vertical retinal profiles of both eyes. Open circles represent orange light and closed circles blue light. The inverted configurations are characteristic of retinitis pigmentosa.

strain, a common mode of inheritance of retinitis pigmentosa (Falls, 1950, 1955; Bell, 1922).

CASE 4.

Mrs. J. I. M. was 54 years of age with 20/20 corrected vision in each eye. She had been night blind for about 11 years, and recently had trouble avoiding objects. The typical bone corpuscle-shaped pigment deposits were found in the nasal halves of both fundi, beginning about two disc diameters from the optic disc. The temporal halves of the fundi appeared normal and free from pigment. Visual fields (fig. 13) showed equal and symmetrical defects temporally. These tended to envelop the fixation point extending across the midline into the nasal fields between 10 degrees and 25 degrees both above and below the foveas. The fields were measured with a three-mm. white test object.

The retinal profiles (fig. 14) displayed the usual inverted form characteristic of retinitis pigmentosa. All retinal function had disappeared beyond 10 degrees nasally in the right eye, and beyond 15 degrees nasally in the left eye. Complete loss of all function had also occurred 20 degrees above and below both foveas. Within a radius of 25 degrees of the foveas no rod vision could be measured, rods being either completely absent or very severely damaged. The fovea was normal as in the previous cases, and a normal complement of cones seemed to be present in a few places two degrees parafoveally. Elsewhere, cone vision also had suffered. In the far periphery, some rod vision was present, but with pathologically high thresholds. Dark-adaptation measurements 10 degrees below the fovea of the right eye (fig. 15) revealed a pure cone curve, with thresholds displaced somewhat upward. Dark adaptation 30 degrees above the fovea of the right eye revealed both rod and cone function with all thresholds abnormally high. The lesions in this patient were obviously more advanced than those in the preceding three cases. Again, the retinal profiles corresponded very well with the visual fields.

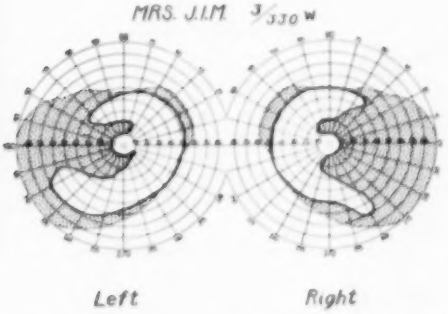

Fig. 13 (Zeavin and Wald). Case 4. Visual fields measured with a three-mm. white test object. Equal and symmetrical defects appear temporally.

CASE 5

Several more advanced cases of retinitis pigmentosa were studied, all with so-called gun-barrel vision. All of them displayed only small round central fields extending to about 10 degrees to 20 degrees from the fixation point. Retinal profiles of these patients were all alike. (fig. 16, K. F.), and displayed the typically inverted configuration. The foveas still appeared to be entirely normal in these advanced cases. The cones two degrees parafoveally also seemed undamaged. No retinal elements seemed to be functional beyond a radius of 10 to 20 degrees from the foveas. Within this radius, only cone vision was measurable, and that was damaged everywhere beyond two degrees parafoveally. As a result, the retinal profiles took on the shape of parabolas opening upward.

CASE 6

H. A. was 51 years of age with 20/15 corrected vision in the right eye and 20/70 in the left eye. He had been night blind as long as he could remember. His fundi had the typical bone corpuscle-shaped pigment deposits in the extreme periphery of the nasal retinas of both eyes. The fields, measured with a three-mm. white test object, were the same in both eyes (fig. 17). They were circular in shape, with their limits at a radius of 35 to 40 degrees around the fixation points.

At the age of 30 years, this patient's fields had been full and normal. During the next eight years, his fields contracted, a scotoma splitting off an island of visual field in the extreme temporal periphery of both eyes. Then this island also disappeared, leaving the fields in their present contracted state. They have remained the same for the past 10 years.

The retinal profiles (fig. 18) displayed a perfectly normal cone profile (fig. 6) extending out to the 40-degree radius tested by us. This meant that the retina was functionally rod-free, with a full complement of undamaged cones throughout, in our experience a unique condition. Compared with the measurements of visual fields, the retinal profiles revealed a slightly larger area of functional retina. The general configuration of the curves did not resemble those described above, although the cone profile was slightly inverted, as in the normal eye. In all the other cases of retinitis pigmentosa we have studied, cone as well as rod vision was at least partially damaged in the periphery; whereas here rod vision had deteriorated completely, leaving cone vision apparently unaltered.

IV. DISCUSSION

The object of these experiments was to provide an accurate and meaningful description of the functional state of the retina in retinitis pigmentosa, to serve as an index of the progress of the disease, and the efficacy of any treatment that might be undertaken.

From this point of view the type of measurement we call a retinal profile furnishes a cross-section of rod and cone function, the functional counterpart of a histologic cross-section. Since by microscopic examination the layer of rods and cones appears to be the first to suffer in this disease (see Cogan, 1950), such a functional measurement prob-

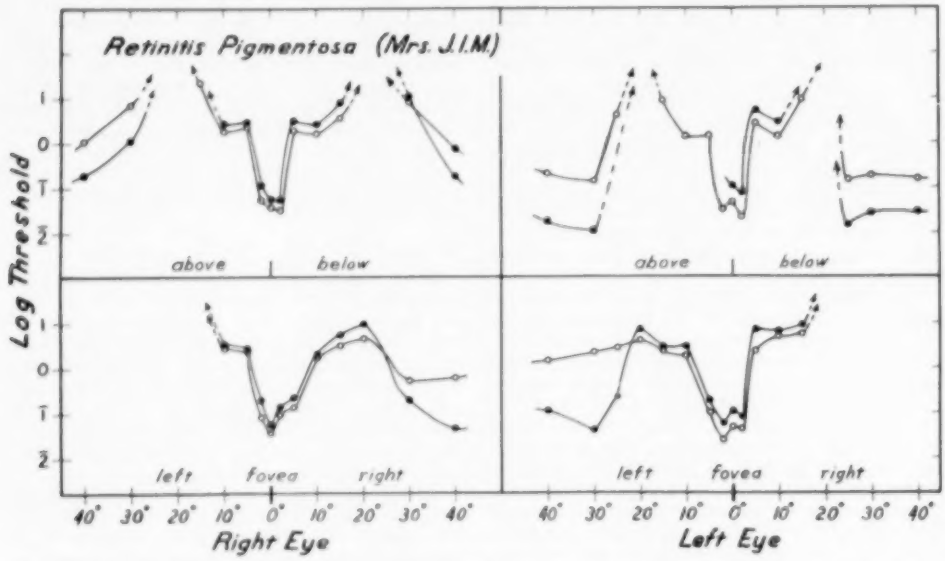

Fig. 14 (Zeavin and Wald). Case 4. Horizontal and vertical retinal profiles of both eyes. Open circles represent orange light and closed circles blue light. All profiles display the inverted form characteristic of retinitis pigmentosa. All retinal function has ceased beyond 10 degrees nasally in the right eye, and beyond 15 degrees nasally in the left eye. There is also complete loss of function 20 degrees above and below each fovea. Only the characteristics of cone vision are retained in the functional areas within 25 degrees of the foveas.

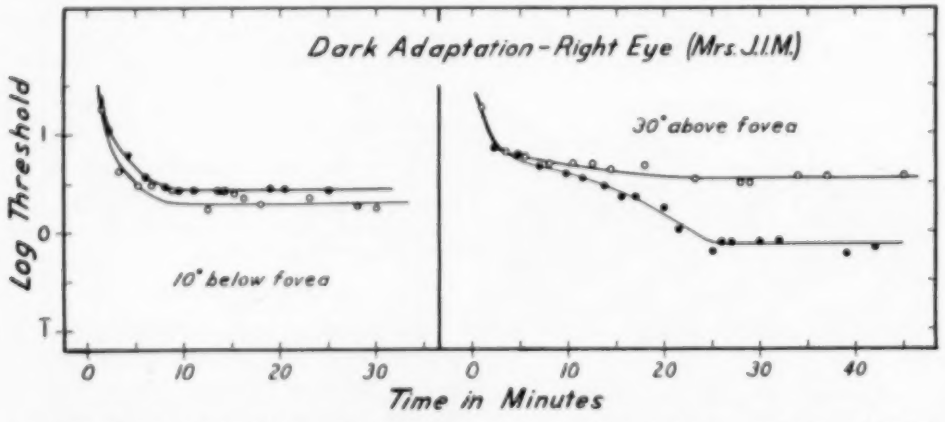

Fig. 15 (Zeavin and Wald). Case 4. Dark-adaptation curves. Open circles represent orange light and closed circles blue light. Ten degrees below the right fovea, a pure cone curve is obtained, with thresholds somewhat higher than normal. Thirty degrees above the right fovea, both rods and cones are functional, both abnormally high in threshold. These curves are consistent with the retinal profiles.

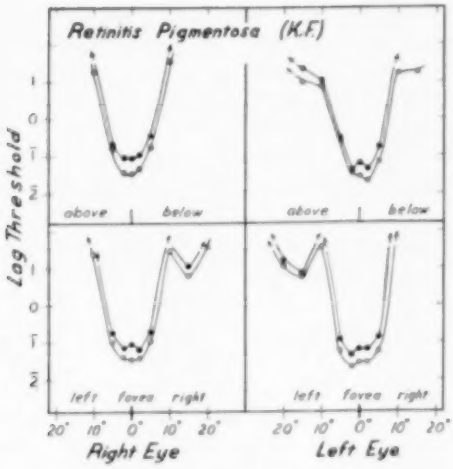

Fig. 16 (Zeavin and Wald). Case 5. Horizontal and vertical retinal profiles in an advanced case of retinitis pigmentosa with gun-barrel fields. Open circles represent orange light and closed circles blue light. No retinal function appears beyond 10 degrees from the foveas, except temporally, where some vision persists out to 20 degrees from the foveas. Only cone vision is in evidence. Even in this advanced stage, the foveas appear perfectly normal. The inverted configuration is typical of retinitis pigmentosa, the profiles taking in this extreme case the form of parabolas opened upward.

ably comes close to following the course of the primary lesion.

Even in early stages of the disease, the entire extra-foveal retina is abnormal; yet pigment deposits appear only in the more degenerated areas. This indicates that the condition, retinitis pigmentosa sine pigmento, probably characterizes every case of the

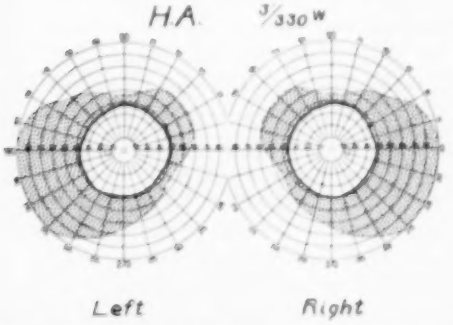

Fig. 17 (Zeavin and Wald). Case 6. Peripheral fields measured with a three-mm. white test object.

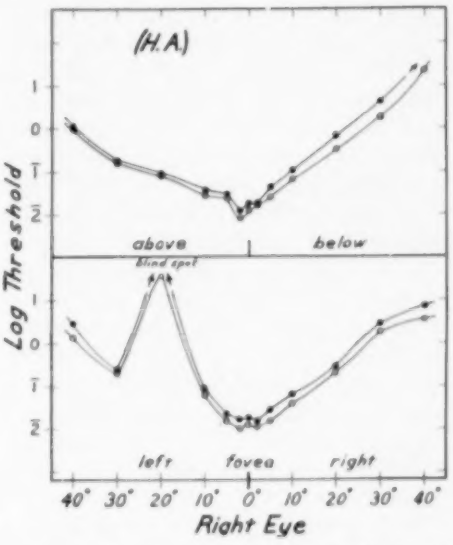

Fig. 18 (Zeavin and Wald). Case 6. Horizontal and vertical retinal profiles of the right eye. The profiles appear normal for cone vision throughout. The retina displays no rod function at all. Open circles represent orange light and closed circles blue light. The horizontal profile happens in this instance to pass through the optic disc.

disease at the beginning. Indeed, unless the entire fundus is occupied with pigment deposits, it follows that every case is partly "sine pigmento." Apparently pigment is deposited only when the visual elements of the retina have largely or completely degenerated.

Initially the disease seems to attack all the rods, especially those within 25 degrees from the fovea, the area most severely hit at this stage. The rod degeneration is first completed within this area, and later more peripherally. It is not clear whether in the same area cones begin to degenerate simultaneously with the rods or later. Certainly the peripheral cones degenerate, but always outlast the rods everywhere. The fovea, together with the cones in the surrounding few degrees of retina, appeared in our cases to remain relatively normal even in late stages of the disease when only gun barrel fields remain. They differ in this regard from most of the cases reported by Mandelbaum

(1941) and by Haig and Saltzman (1955).

That retinitis pigmentosa involves hereditary factors has long been recognized. Its inheritance follows no simple pattern; cases have been described in which it appears dominant, recessive, or as a sex-linked recessive (Bell, 1922; Falls, 1950, 1955). Our three siblings (cases 1, 2, and 3) appear to involve a dominant trait. What is most remarkable is the great similarity in the pattern of retinal degeneration exhibited by these three persons. The patterns presented by the two brothers are so nearly identical as to be easily confused (figs. 8 and 11); and that of the sister, though less advanced, is strikingly similar (fi. 12). The retinal profiles in these cases are almost like a family signature.

It is no news that rod vision deteriorates more rapidly than cone vision in retinitis pigmentosa. There is apparently a wide latitude in this differential. We cannot be sure that our Case 6 is necessarily to be classed as an example of this disease, rather than some other type of retinal degeneration associated with night blindness. It offers the peculiar picture of an apparently complete loss of rod vision associated with apparently normal cone vision. Whatever mechanisms are invoked to explain the etiology of retinitis pigmentosa must attempt to account for large differentials in the susceptibilities of rods and cones.

In this connection it may be of interest to pursue a suggestion, made several times in the literature, that retinitis pigmentosa is connected with some local failure in the supply of or in the ability to use vitamin A (Yudkin, 1933; Cogan, 1950). The lesions at one stage of vitamin A deficiency resemble somewhat those in this disease. The layer of rods and cones is the first to deteriorate in vitamin A deficiency; and such deterioration is characteristic of retinitis pigmentosa. In more advanced vitamin A deficiency, however, the inner retinal layers also succumb, while apparently remaining intact in retinitis pigmentosa (Cogan, 1950).

Recent observations on the biochemistry of rod and cone vision have a bearing, both upon the possibility of a local failure in vitamin A metabolism, not apparent elsewhere than in the eye tissues, and upon the greater susceptibility of rods than cones (see Wald, 1955). It has lately been shown that both rod and cone vision depend upon a specific *cis* isomer of vitamin A, called neo-b (Hubbard and Wald, 1952-53; Wald, Brown, and Smith, 1954-55). This has a hindered *cis* configuration, and probably always requires special conditions for its formation (Wald, Brown, Hubbard, and Oroshnik, 1955). The *neo-b* isomer of vitamin A has so far been identified only in eye tissues where an enzyme has also been found that assists in its formation (Hubbard, 1956-57). We have not yet succeeded in finding any neo-b vitamin A in livers or in mammalian blood plasma. One can imagine therefore a condition in which the general supply of vitamin A to the organism and its general metabolism are wholly adequate, yet a failure to form this specific isomer might result in visual failure.

The second point is equally pertinent. The visual pigments, rod and cone, are synthesized by the union of vitamin A, in the form of its aldehyde retinene, with specific proteins of the rods and cones, the opsins. Cone opsin unites with neo-b retinene with enormously greater speed than does rod opsin. As a result, the synthesis of the cone pigment is complete when that of the rods has hardly begun (Wald, Brown, and Smith, 1954-55). If therefore the supply of neo-b vitamin A or retinene were severely limited, the cones would get it all. In this way the cones might maintain their function relatively unimpaired under conditions in which rod function had virtually ceased.

What connection might there be between vitamin A deficiency and the *anatomic* degeneration of the rods and cones? This is not entirely a fair question, for in vitamin A deficiency tissues in many parts of the body deteriorate for reasons not yet understood in any instance. Yet something may be said in this connection specifically involving the

outer segments of the rods and cones. In the outer segment of a rod, rhodopsin is an important element of structure. It accounts for about 40 percent of the dry weight of the outer segment in the frog, or about 60 percent of the protein; and about 14 percent of the dry weight in cattle, or about 22 percent of the protein.

We have found lately that rod opsin is stabilized by its attachment to retinene, as in rhodopsin (Radding and Wald, 1955-56). If the supply of neo-b retinene were to fail, so that opsin could not form rhodopsin, it is probable that opsin itself would soon deteriorate, and with it the structure of the outer segments of the rods.

The cones are in a different position. The rapidity with which cone opsin couples with neo-b retinene ensures that under conditions of deficiency the cones will capture whatever neo-b vitamin A remains available. In addition cone visual pigments, being much more dilute than those of the rods, play a proportionately small *structural* role compared with rod pigments. For both reasons one might expect the cones to survive, functionally and anatomically, after the rods had failed.

All of this, however, is relatively unsupported hypothesis. Recently Noell (1953) has observed retinal lesions in monoiodoacetate poisoning which he believes comparable with those of retinitis pigmentosa. The etiology of this disease is still as mysterious as ever. One can only hope that some increased understanding of it will come out of the growing knowledge of retinal structure and biochemistry, and that such methods of observation as we have described will help in this progress.

Summary

Visual thresholds and adaptation of rods and cones have been studied in normal persons and cases of early and late retinitis pigmentosa. A special perimetric adaptometer was designed with which thresholds can be measured foveally and in any quadrant of the retina within a radius of 40 degrees. Thresholds were measured alternately in blue and orange lights, so chosen that in cone vision the threshold for blue lay *above* that for orange, in rod vision well *below* that for orange. In this way one could specify unequivocally rod or cone function, or any mixture of the two.

Visual thresholds, measured across various meridians of the dark-adapted retina, yield cross-sections ("profiles") of rod and cone function within the areas of measurement. In normal persons such profiles form a peak at the fovea, descending rapidly toward the periphery. The thresholds also shift from cones to rods in a transition zone 0.5 to 2.0 degrees around the fixation center. In all our cases of retinitis pigmentosa the profile is inverted, the foveal thresholds forming a minimum. Associated with this change, rod vision has greatly deteriorated, most and earliest in the region within 25 degrees of the fovea, eventually also more peripherally. Cone vision also deteriorates peripherally, though much more slowly; it may appear normal in the fovea and immediate parafovea even in advanced stages of the disease, when gun barrel vision alone is present. Pigmentation of the retina is apparent only after visual function has declined greatly.

Three siblings, two diagnosed as having retinitis pigmentosa, the third with no clinical history, all displayed very similar retinal profiles. One atypical case exhibited normal cone vision over the whole retina, though rod vision had deteriorated completely.

16 Divinity Avenue (38).

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Discussion

Dr. Harold Falls (Ann Arbor, Michigan): Dr. Zeavin and Dr. Wald aptly have applied the visual threshold and dark-adaptation procedures as aids in the differential diagnosis of certain varieties of of the disease, retinitis pigmentosa. This is a most important contribution and will be a valuable adjunct to the clinician in the study of such affected individuals and families. It is to be anticipated that such detailed examinations will permit more accurate diagnosis and classification.

Personal clinical observation has led me to conclude, as has been stressed by Dr. Burian, that the term "retinitis pigmentosa" is a nomenclature wastepaper basket. Several specific types of retinal pigmentary degeneration exist:

1. A primary neuroepithelial degeneration (so-called abiotrophy). The pigmentary changes follow loss of function particularly of the rods.

2. Choroidal atrophy (degeneration) with slow but inevitable secondary degeneration of both rods and cones. Night blindness is quite late in this type. This type perhaps may be secondary to the choriocapillaris changes so capably exhibited by Dr. Wolter. It is possible that even pigmentary alteration may precede loss of function of the neuroepithelial layer.

Careful dark adaptation and electroretinographic studies will usually disclose evidence that both the rods and cones are seriously affected (eventually). In the primary degenerative variety it is chiefly the rods (at first) that are extensively defective. As the toxic or enzymatic and degenerative process continues the cones also are affected.

In respect to the term abiotrophy recent studies (Sorsby, et al.) suggests that these tissues may be congenitally atypical either anatomically or physiologically.

Dr. Burian has correctly stressed that the gene and enzyme function are intimately interwoven and in this I wish to concur. We should therefore begin to search for the abnormal enzymatic functioning in these diseases.

To further emphasize my original point I wish to call to your attention that the following types of inheritance patterns are known for the entities clinically termed "retinitis pigmentosa:" (1) autosomal dominant, (2) autosomal recessive, (3) sex-linked recessive, (4) sex-linked intermediate, (5) partial sex-linked dominant, and (6) partial sex-linked recessive.

When so many known varieties exist it certainly behooves us to apply all available diagnostic aids.

The clinician certainly will welcome the help of the experimental laboratory.

DR. HERMANN BURIAN (Iowa City, Iowa): It has been pointed out (Louise L. Sloan, Am. J. Ophth., **33**:1077 [July] 1950) that routine perimetric examinations with the white test objects is inadequate for the study of pathologic conditions in which primarily one retinal mechanism is affected. It is, therefore, to be welcomed when studies are carried out in such patients which aim specifically at the investigation of the different retinal mechanisms.

In the paper which we have just heard the authors have presented us with their highly interesting findings in a complex and difficult subject, retinitis pigmentosa. They have not only confirmed the inverted form of the retinal profiles obtained by perimetric adaptometry, but have also demonstrated very strikingly the value of colored stimulus lights for the differentiation of the functional status of the rods and cones.

These findings are valuable and important and should stimulate us to extend similar investigations to other pathologic conditions of the retina. I think here especially of the so-called inverse type of retinitis pigmentosa, of choroideremia, of pseudoretinitis pigmentosa, and the like. But what has fascinated me most in the paper is the hint, given us by the authors, as to what might be the fundamental cause of retinitis pigmentosa. They speculate that it might be due to a limitation in the supply of the neo-b isomer of vitamin A, for the formation of which a specific enzyme is required. If neo-b vitamin A were produced in insufficient quantity it would be speedily used up during dark adaptation in the synthesis of the cone pigments, leaving the rods without the necessary supply.

This view fits in very well with the current thoughts about the abiotrophies of the retina and might also explain the gradual onset and progress of the disease. Elwyn (Arch. Ophth., **53**:619, 1955) says that in the concept of heredodegeneration there is the implication that the genes which have to do with specific enzymatic activities of certain cells are defective. At first these genes exert their activity properly, but after a variable period of time, measured in years, they lose their control. This leads to the reduction in the specific enzymatic activities and finally to the premature death of the cell. In retinitis pigmentosa the specific enzyme involved might well be the one which has to do with the formation of neo-b vitamin A.

A paper which brings such provocative new thoughts about the pathophysiology of retinitis pigmentosa cannot fail to raise certain questions. Indeed, this is one of its purposes. One wonders why the disease begins in such a circumscribed retinal area. One wonders where the other clinical manifestations, the narrow blood vessels, the frequent appearance of a complicated cataract, fit into the picture. One also wonders whether and how the reasoning of the authors could be applied to conditions in which there is a primary heredodegeneration of the cones.

These questions are not addressed to the authors for an immediate answer. They are rhetorical questions. As an ophthalmologist I can foresee much fruitful work with clinical cases, stimulated by the paper of the authors, which should help us toward a better understanding of the mechanisms underlying these mysterious diseases. As an ophthalmologist interested in the physiologic aspects of our specialty I feel confident that such studies, in turn, will enrich our knowledge of the physiology of the visual mechanism.

THE DEVELOPMENT OF THE ORBITAL FASCIA

WALTER H. FINK, M.D.
Minneapolis, Minnesota

The development of the orbital fascia is of interest to both the anatomist and the physiologist.

This knowledge is important from the anatomic standpoint because it gives a broader basis for understanding the mature anatomy.

It is important from the physiologic standpoint because in tracing the different stages of development of the orbital fascia we acquire a broader concept of the physiologic function.

The literature reveals very little as to the development of the orbital fascia in the human. Most of the material available relates to lower forms and deals with the microscopic study of the very early stages of development of the globe, muscles, and blood vessels. The orbital fascia receives little attention, being casually referred to in describ-

ing the early development of the other structures. We have, therefore, at the early stage a very incomplete picture of the orbital fascia development.

Data related to the later stages of orbital fascial development are even more incomplete. Between the initial stages and the mature development a wide gap exists in data relating to the orbital fascia. In order to obtain additional data covering this later period of development and thus trace the development in its entirety, I studied a series of specimens at various stages of development from the 40-mm. stage to maturity.

In the presentation the material will be divided into two sections:

1. The development of the orbital fascia to the 40-mm. stage, and is based on available data found in the literature.

2. The development from the 40-mm. stage to maturity, and is based on material obtained from a study of a series of specimens at various stages of development.

1. The development of the orbital fascia to the 40-mm. stage

According to Mann,[1] the early stages of development of the orbital fascia are as follows: The mesoderm adjacent to the primary optic vesicle is at first entirely undifferentiated. It forms a loose meshwork of stellate cells connected by interlacing processes.

At the three- to four-mm. stage some differentiation is present which relates to blood cells. Nucleated erythrocytes can be recognized and can be seen to lie in spaces in the mesoderm meshwork which are lined by a definite wall formed of a single layer of flattened mesoderm cells (endothelium). We can therefore distinguish two types of mesoderm at this stage, vascular and stromal. The stromal mesoderm in the neighborhood of the optic vesicle can be recognized as belonging to the two main mesodermal masses, namely, paraxial mesoderm, immediately surrounding the optic vesicle and in contact with it, and visceral (maxillary) mesoderm, lying below this.

Both types of mesoderm are vascularized. They are similar in appearance, but slight differences of texture and a faint line of vascular condensation, where they lie in contact, serve to distinguish them.

The mesoderm immediately and completely surrounding the optic vesicle, and its stalk, belongs to the paraxial layer and is continuous with that in which the brain is embedded. It rests below and behind on the (visceral) maxillary mesoderm.

The orbit with its related structures is formed from this surrounding mesoderm. From the paraxial mesoderm are developed the structures within the orbit, namely, the blood vessels supplying the eye, the extrinsic muscles, the ciliary muscle, the sclera, the sheath of the optic nerve, the various connective tissue structures in the orbit, and nearly the whole of the inner and upper bony wall.

The visceral mesoderm (maxillary process) is mainly concerned with the formation of the bones of the lower and outer walls of the orbit. Its growth also plays an important part in the mechanism by means of which decrease in the angle between the optic axes is brought about.

The paraxial mesoderm from which the orbital fascia and extrinsic muscles develop represents the fusion of persisting parts of the three of the most rostrally located of the head somites of primitive forms. The three distinct but closely approximated anlagen represent the earliest changes.

Gilbert[2] has shown that the paraxial mesoderm of the human embryo develops into a pair of premandibular condensations of prechordal plate origin situated adjacent and caudal to the optic vesicles and connected across the midline by a solid bridge of prechordal mesoderm.

Several small epithelium-lined cavities appear early in each premandibular condensation. These transitory head cavities of the human embryo, which persist for a short time only and never coalesce, may be regarded as "abortive attempts" to form a pair of larger premandibular head cavities,

such as are found in many marsupials, birds, reptiles, and elasmobranches.

From the first head somite (premandibular condensation) muscle cells are formed which grow toward the eye and gain attachment to it. There are four main outgrowths from this condensation corresponding to the rudiments of the superior, inferior, and internal recti and the inferior oblique, namely, those muscles which are supplied by the oculomotorius, the nerve of supply to this head segment.

From the second head somite (mandibular condensation (comes the rudiment of a single eye muscle, the superior oblique, the nerve to this, being the trochlearis, while the third head somite gives rise to the lateral rectus muscle and has the abducens as its segmental nerve.

In 7.5-mm. embryo the condensation of the paraxial mesoderm is fairly well defined and appears as a spindle-shaped structure medial to the optic vesicle. There is at first no separation between the individual condensations which later develop into the various extrinsic muscle and fascia.

At the nine-mm. stage the condensation shows signs of cleavage. The differentiation is first apparent at the muscle insertion and gradually spreads backward. The individual muscle bellies can be just distinguished by the 14-mm. stage and the four recti and the two oblique can be recognized as distinct muscles at the 20-mm. stage. By the 40-mm. stage the insertions and muscle bodies show a definite separation from the surrounding mesoderm although the posterior third in the region of the muscle origins continued to be indistinct, blending with the surrounding mesoderm.

During this early period there is no evidence of change in the mesoderm which is destined to become the sheaths of the muscles, tenons fascia, and the other fascial membranes of the orbital fascia.

As near as can be determined, the first evidence of mesodermal condensation other than that of the extrinsic muscles relates to the formation of the sclera. The sclera differentiates by condensation from the mesoderm encircling the optic cup. In the earliest stages, up to 18 mm., there is no distinction of layers in the mesoderm in which the cup is embedded. Soon after, the cells of the mesoderm nearest the cup tend to become arranged with their long axes parallel to its wall. Almost immediately (19 to 20 mm.) some indication of a condensation appears, running from the region of future attachment of the extrinsic muscles forward to the angle of the anterior chamber. Behind the equator there is no trace of a line of demarcation between choroid, sclera, or orbital tissue.

The extrinsic muscles can be recognized at this stage forming a sort of funnel around the eye, and the sclerotic rudiment blends with this and fades away along it. By 35 mm. a very faint condensation, some little distance away from the eye, can be seen at the posterior pole and, by 65 mm., this has increased and approached nearer to the choroid. Thereafter, development of the posterior part of the sclera proceeds, and by five months the sclera is well differentiated all the way around.

The capsule of Tenon, like the sclerotic, is first recognizable in its anterior part. The rudiment of that portion of it which is related to the insertions of the recti muscles is recognizable at 80 mm. as a thin sheet of differentiating connective tissue. The posterior part is not recognizable in its full extent until the middle of the fifth month (240 mm.).

2. Development from the 40-mm. stage to maturity

Because of the lack of available information regarding the development of the orbital fascia following the initial stages, I considered it necessary to study a series of orbits at different stages of development.

The specimens examined by me were selected from a larger group and represent as near as could be determined the following

stages of development: 40 mm., 75 mm., 140 mm., 260 mm., 375 mm., 450 mm., 500 mm. (birth), two years of age, 11 years, and the mature eye. (The measurements represent the crown-to-heel length.)

The series was started at the 40-mm. (two and one-half month) stage for three reasons: (1) Embryonic developments previous to the 40-mm. stage have been extensively studied and we have a fairly complete picture of the early embryonic stage; (2) the development following this early embryonic stage has not been completely studied and certain gaps in our knowledge still exist; (3) the 40-mm. stage is the earliest stage possible to dissect satisfactorily. The early specimens were dissected under water using magnification. Although certain phases of the anatomy could not be clearly determined in the early specimens they revealed certain data which seem significant.

The study of the various specimens revealed the following:

A. 40-MM. (TWO AND ONE-HALF MONTH) SPECIMEN (fig. 1)

The diameter of the eyeball was one mm. The angle between the optic axes was about 70 degrees.

The length of the muscle cone could not be determined. The muscles, although poorly defined, could with care be recognized especially at the point of insertion. The muscle bodies were pale and had much the same appearance as the surrounding mesoderm with which they blended without a sharp line of demarcation. The origin third of the muscle cone could not be satisfactorily dissected as it seemed to merge with the surrounding mesoderm. The rectus muscles were inserted at the equator of the globe and the insertion points were fairly well defined. These muscles were proportionally thick and short as compared with the later stages.

The obliques could be defined but like the recti blended with the surrounding tissue. Their insertions seemed to blend with the adjacent rectus insertion. Their angulation in relation to the globe appeared to be between 40 degrees to 45 degrees. The superior oblique tendon was broad in proportion to its length as compared with its later development.

There was no evidence of condensation of the mesoderm in relation to the muscles but seemed to blend with the muscle tissue with

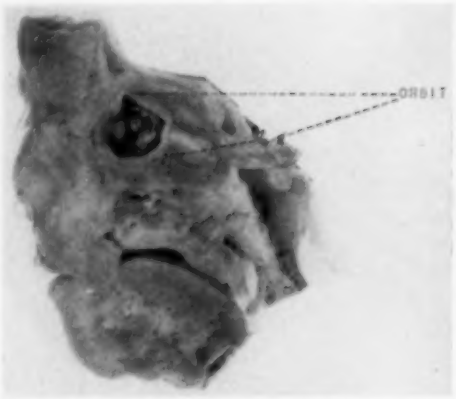

Fig. 1 (Fink). Photograph of a 40-mm. (two and one-half month) specimen magnified three times.

The length of the muscle cone could not be determined. The muscles, although poorly defined, could with care be recognized especially at the point of insertion. The muscle bodies were pale and had much the same appearance as the surrounding mesoderm with which they blended without a sharp line of demarcation. The origin third of the muscle cone could not be satisfactorily dissected as it seemed to merge with the surrounding mesoderm. The rectus muscles were inserted at the equator of the globe and the insertion points were fairly well defined. These muscles were proportionally thick and short as compared with the later stages.

There was no evidence of condensation of the mesoderm in relation to the muscles but seemed to blend with the muscle tissue with no definite line of demarcation. This was especially so near the posterior third of the orbit where the various muscle origins could not be distinguished. The only condensation of the mesoderm noted was in connection with the optic cup where the mesoderm was condensed to make up the scleral covering. This was evident on the anterior portion of the optic cup. The posterior section of the optic cup showed little differentiation of the mesoderm and seemed to be continuous with the undifferentiated mesoderm surrounding the area.

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B. 75-MM. (THREE-MONTH) SPECIMEN (fig. 2)

The diameter of the globe was three mm. The angle between the optic axes was about 65 degrees.

The rectus muscles were fairly well defined and although they merged with the surrounding tissue they could be readily separated. The muscle cone was about five mm. long, and the points of origin of the muscles were indefinite although much less so than in the previous specimen. The muscle bodies were proportionally thicker in comparison to their length than in later specimens. The insertions were near the equator and could be easily determined.

The obliques were fairly well defined and like the recti merged with the surrounding tissues. Their insertions could be accurately determined and seemed to blend with the adjacent rectus. With careful dissection it was evident that the lines of insertion were posterior to the rectus insertion.

The oblique insertions seemed nearer the equator than in the adult eye. The angle of the oblique muscle planes with the anterior-posterior vertical plane of the eye was about 45 degrees. The reflected portion of the superior oblique was almost round and tendinous in appearance. There was an acute angle (30 degrees) between the two parts of the superior oblique.

The mesoderm at this stage showed definite evidence of early condensation in the vicinity of the muscles and especially near the insertions. The individual insertions could be more clearly defined and a more definite separation and thickening of the adjacent mesoderm was noted.

The mesoderm in the posterior third of the orbit showed less differentiation as compared with the anterior third. Some condensation of the mesoderm adjacent to the developing sclera was noted indicating the early formation of the Tenon's fascia. This

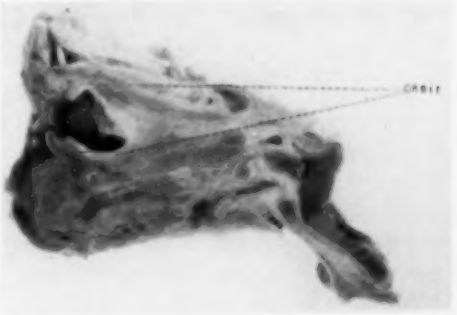

Fig. 2 (Fink). Photograph of a 75-mm. (three-month) specimen magnified three times.

The rectus muscles were fairly well defined and, although they merged with the surrounding tissue, they could be readily separated. The muscle cone was about five-mm. long and the points of origin of the muscles were indefinite although much less so than in the previous specimen. The muscle bodies were proportionally thicker in comparison to their length than in later specimens. The insertions were near the equator and could be easily determined.

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The mesoderm in the posterior third of the orbit showed less differentiation as compared with the anterior third. Some condensation of the mesoderm adjacent to the developing sclera was noted indicating the early formation of the Tenon's fascia. This was especially noticed near the muscle insertions.

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C. 140-MM. (FOUR-MONTH) SPECIMEN (fig. 3)

The diameter of the globe was six mm. The angle between the optic axes was about 65 degrees. The length of the muscle cone was about 10 mm. The muscles were somewhat narrower in proportion to their length than seen in the previous specimens.

The origins of the recti were well defined and their insertions encircled the equator of the globe. The muscle bodies were sharply defined and easily separated from the surrounding tissue.

The oblique muscle planes made an angle of about 45 degrees with the anterior-posterior vertical plane of the globe. The two parts of the superior oblique formed a less acute angle (about 35 degrees). The reflected portion of the superior portion of the superior oblique was well defined and cordlike in appearance. The oblique insertion showed a more definite separation from the adjacent rectus and could be more easily traced to its insertion just posterior to the equator.

The condensation of the mesoderm related to the extrinsic muscles was more evident than in the previous specimen and was especially so near the muscle insertions. Tenon's fascia was more definite. Early signs of differentiation of the supporting reticulum were noted. There were small scattered areas of fat in the supporting reticulum.

Fig. 3 (Fink). Photograph of a 140-mm. (four month) specimen magnified two times.

The origins of the recti were well defined and their insertion encircled the equator of the globe. The muscle bodies were sharply defined and easily separated from the surrounding tissue.

The condensation of the mesoderm related to the extrinsic muscles was more evident than in the previous specimen and was especially so near the muscle insertions. Tenon's fascia was more definite. Early signs of differentiation of the supporting reticulum were noted. There were small scattered areas of fat scattered in the supporting reticulum.

D. 260-MM. (FIVE AND ONE-THIRD MONTH) SPECIMEN (fig. 4)

The diameter of the globe was 10 mm. The angle between the optic axes was about 65 degrees. The length of the muscle cone was about 14 mm.

The muscles were more clearly separated than in the previous specimens and were longer and narrower in proportion than previously seen. The points of origin were more evident. The rectus insertions were better defined and encircled the globe about the equator.

The oblique planes made an angle of about 45 degrees with the anterior-posterior vertical plane of the globe. The superior oblique showed an angle of about 45 degrees between its two parts. Its reflected tendon was better defined and its insertion could be clearly made out posterior to the equator but seemingly more anterior than in the mature eye. The inferior oblique likewise showed a more defined insertion and also not so far posterior as the adult eye.

Condensation of the mesoderm was more definite than in the previous stage. This was especially noted in the region of the origin and insertion of the muscles. There seemed to be a definite but poorly defined sheath surrounding the anterior half of the muscle bodies.

The condensation of mesoderm was definite surrounding the eyeball although more pronounced in the anterior half near the insertion of the muscles.

Fig. 4 (Fink). Photograph of a 260-mm. (five and one-third month) specimen magnified two and one-fourth times.

The muscles were more clearly separated than in the previous specimens and were longer and narrower in proportion than previously seen. The points of origin were more evident. The rectus insertions were better defined and encircled the globe at about the equator.

Condensation of the mesoderm was more definite than in the previous stage. This was especially noted in the region of the origin and insertion of the muscles. There seemed to be a definite but poorly defined sheath surrounding the anterior half of the muscle bodies.

The condensation of mesoderm was definite surrounding the eyeball although more pronounced in the anterior half near the insertion of the muscles.

The supporting reticulum was in evidence and some fat was present in the supporting reticulum.

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E. 375 MM. (SEVEN AND ONE-THIRD MONTH) SPECIMEN (fig. 5)

The diameter of the globe was 13 mm. The muscle cone was about 18-mm. long. The muscles were well defined and were longer and narrower than in the previous specimen. The origins and insertions of the recti were more evident and the insertion end of the muscles took on a more definite tendinous appearance.

The oblique muscle planes made an angle of about 45 degrees with the anterior-posterior vertical plane of the globe. The superior oblique showed an angle of about 45 degrees between its two parts. The superior oblique tendon was round and tendinous in appearance and inserted slightly more posterior to the equator than described in the previous specimen. The inferior oblique insertion was also more evident and more posterior to the equator than in the previous specimen.

The fascial development was more advanced. Tenon's fascia was well defined es-

Fig. 5 (Fink). Photograph of a 375-mm. (seven and one-third month) specimen magnified two times.

The muscles were well defined and were longer and narrower than in the previous specimen. The origins and insertions of the recti were more evident and the insertion end of the muscles took on a more definite tendinous appearance.

The fascial development was more advanced. Tenon's fascia was well defined, especially that portion surrounding the muscle insertions. Fascial formation was evident surrounding the optic nerve. The muscle sheaths and expansions were easily identified. The supporting reticulum and the periorbita were more definitely defined. The orbital fat was more evident.

pecially that portion surrounding the muscle insertions. Fascial formation was evident surrounding the optic nerve. The muscle sheaths and expansions were easily identified. The supporting reticulum and the periorbita was more definitely defined. The orbital fat was more evident.

F. 450-MM. (NINE-MONTH) SPECIMEN (Fig. 6)

The diameter of the eyeball was 15 mm. The muscle cone was about 21 mm. long. The muscles presented much the same picture as previously described. They were somewhat better defined and more easily separated from the surrounding tissue. Also they were proportionally longer, less broad, and thinner. The origins and insertions of the recti were more defined and took on more the appearance as seen in the mature development. The oblique planes were as described in the previous specimen and the angle between the two portions of the superior oblique was less acute (about 50 degrees). The insertions of the obliques were more defined and somewhat more posterior.

The fascial development was more advanced than in the previous stage showing a greater differentiation of the muscle sheath, Tenon's fascia, and the supporting reticulum.

G. 550-MM. (FULL-TERM) SPECIMEN (fig. 7)

The diameter of the eyeball was 17 mm. Length of the muscle cone was about 25 mm.

The muscles showed the characteristics of

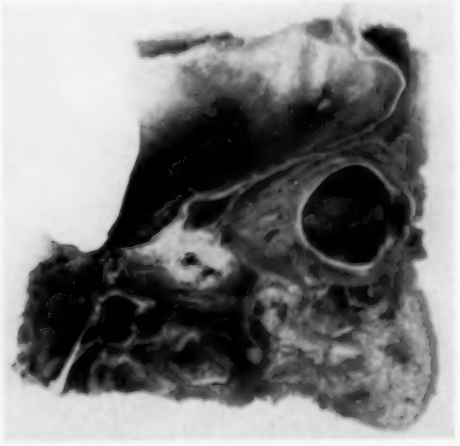

Fig. 6 (Fink). Photograph of a 450-mm. (nine-month) specimen magnified two and one-fourth times.

The muscles presented much the same picture as previously described except that they were somewhat better defined and more easily separated from the surrounding tissue. Also they were proportionately longer, less broad, and thinner. The origins and insertions of the recti were more defined and took on more the appearance as seen in the mature development.

The oblique planes were as described in the previous specimen and the angle between the two portions of the superior oblique was less acute (about 50 degrees). The insertions of the obliques were more defined and somewhat more posterior.

The fascial development was more advanced than in the previous stage showing a greater differentiation of the muscle sheath, Tenon's fascia, and the supporting reticulum.

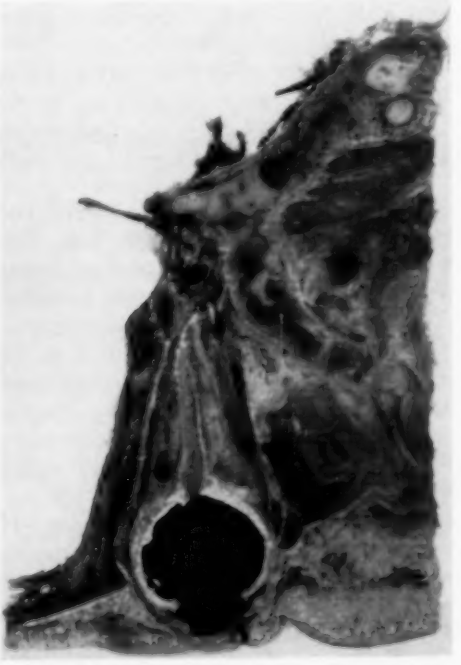

Fig. 7 (Fink). Photograph of a 550-mm. (full-term) specimen magnified two times.

The orbital fascia seemed fully developed although all structures were more delicate in structure than seen in the mature stage. The various fascial muscle sheaths were easily identified and the Tenon's fascia was well developed. The supporting fascial reticulum was less dense and more sharply separated from the fascial sheaths related to the muscles. The fascial covering of the muscles seemed similar in their arrangement to the mature stage.

the mature eye except that they were shorter, somewhat thicker, and broader. The rectus insertions were near the equator.

The oblique planes made an angle of about 45 degrees with the anterior-posterior vertical plane of the globe. The oblique insertions were slightly more posterior than previously described and seemingly the same as found in mature eyes.

The orbital fascia seemed fully developed although all structures were more delicate in structure than seen in the mature stage. The various fascial muscle sheaths were easily identified and the Tenon's fascia was well developed. The supporting fascial reticulum was less dense and more sharply separated from the fascial sheaths related to the muscles. The fascial covering of the muscles surrounding the vertical muscles seemed similar in their arrangement to the mature stage.

H. TWO-YEAR-OLD SPECIMEN

The diameter of the globe was 20 mm.

The picture was similar to that described in the previous specimen, except the muscle cone was about 30-mm. long and the muscles were not so broad or thick. The line of insertion of the recti seemed somewhat more posterior than seen in previous specimens, apparently due to the proportionately greater expansion of the anterior segment of the globe

The fascial structures were similar to the adult in development except for size.

I. 11-YEAR-OLD SPECIMEN (fig. 8)

The diameter of the globe was 22 mm. The muscle development was, in general, similar to the mature eye except that it was proportionally smaller. The muscles were somewhat shorter in proportion to their width than seen in the adult.

The fascial structure had all the appearance of the mature structure.

Fig. 8 (Fink). Photograph of an 11-year-old specimen.

The diameter of the globe was 22-mm. The muscle development of the specimen was, in general, similar to the mature eye except that it was proportionally smaller. The muscles were somewhat shorter in proportion to their width than seen in the adult.

The fascial structure had all the appearance of the mature structure.

J. ADULT SPECIMEN

The diameter of the globe was 22 mm. The muscle development was proportionally larger than in the previous specimen and the muscles were somewhat longer than in proportion to their width. The fascial structure had assumed the mature appearance.

SUMMARY

The presentation traces the development of the orbital fascia from early embryonic life to maturity. The data pertaining to the initial stages are based on the literature and the data from the 40-mm. stage on are based on material obtained by me from a study of a series of specimens at different stages of development.

It is hoped that this study will lead to a better understanding of the anatomy and physiology of the orbital fascia.

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MICROSCOPY WITH A NEW ILLUMINATION TECHNIQUE*

PAUL A. CIBIS, M.D.
Saint Louis, Missouri

INTRODUCTION

The development of the slitlamp improved the study of the surface structures and refractive media in the living eye. One of the most exciting findings of biomicroscopy was the discovery of the internal architecture of the lens. Vogt[1-4] designated these structures as zones of discontinuity or "Abspaltungszonen." Unfortunately the nonuniformity of the lens fiber responsible for the pattern of biomicroscopic discontinuity zones is not apparent on histologic examination. It is the object of this report to describe a simple and versatile illumination technique for the visualization of the zones of discontinuity in routine histologic sections.

TECHNIQUE

The main modalities of microscopic illumination are presented in Figure 1. The essential components of the device used in this study (fig. 2) are a plain-parallel plate (P)** to which a prism (L) is attached. The prism angle should not exceed 45 degrees. The prism base is convex to minimize deflection and to facilitate the entrance of the light emitted from a microscope illuminator or projector into the glass plate P where it travels by internal deflection when the angle of incidence (i) surpasses the critical angle d† at which total reflection occurs. The use of the prism with convex base‡ at the entrance surface of the system proved to provide light of higher intensities than plain edge light.* Focal illumination at the center of the stage can easily be arranged by changes in the distance of illuminator and prism base, thus providing sufficiently high luminance of the microscopic picture to make color photography feasible.

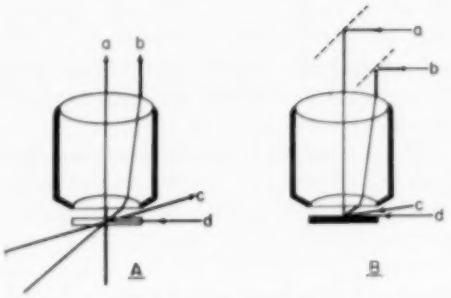

Fig. 1 (Cibis). Diagram illustrating various types of illumination. (A) refers to transparent, (B) to opaque objects; (a) designates axial, (b) oblique, (c) inclined incident, and (d) gazing orthogonal illumination.

The plate-prism combination is placed on the microscopic stage (fig. 4) with the tissue slide in immersion contact by means of distilled water, alcoholic solutions, or glycerin. A 40- or 70-percent solution of ethyl alcohol in distilled water proved to be most satisfactory. For the illumination with transmitted light, the substage illuminator is employed. A separate source is needed for the dark field illumination in the prism-plate system. A most suitable illuminator for that purpose is the Spencer Microlamp "Universal" as shown in Figure 4.

HISTOLOGIC TECHNIQUE

To obtain reproducible and comparable results, a standardized fixation and staining technique is essential. At the present time, it is felt that a 10-percent solution of formaldehyde appears to be a suitable fixative

* From the Department of Ophthalmology and the Oscar Johnson Institute, Washington University School of Medicine. This research was supported in part by the USAF under Contract No. AF 18 (600)-1269; monitored by the USAF School of Aviation Medicine, Randolph Field, Texas.

** 5 by 50 by 170 mm. in dimension, refractive index 1.52.

† $\sin d = 1/u$ whereby u represents the refractive index 1.52.

‡ radius 30 mm.

* Beveled slides for edge light illumination have been patented by Palmer[5] in 1886.

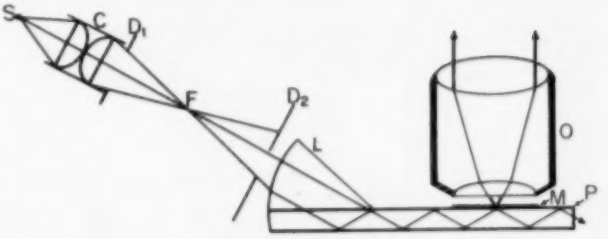

Fig. 2 (Cibis). Arrangement used for unilateral dark-field illumination by light scattering within a plane-parallel glass plate (P) with prism lens (L) fused to its surface. Light source (S), double lens condensor (C), diaphragms (D_1) and (D_2), contact medium (M) with tissue slide, microscope objective (O).

for this purpose. It prevents excessive shrinkage and distortion of the ocular tissue structures; in particular, it preserves the lens structures better than Zenker's and Bouin's solutions. Furthermore, formalin seems to preserve primarily fluorescent substances at their original site and avoids objections against the use of fixatives containing heavy metals in fluorochromic studies. On the other hand, Zenker's and Bouin's solutions are quite better preservatives of the chromatin structure in the nuclei. Continued fixation beyond 24 hours, however, may result in disturbing diffusion of fluorescent material into adjacent tissue components. When primary fluorescence is not the objective, the tissue may remain in the formalin solution for 48 hours or more. Thereafter, it is washed in running tap water and subsequently passed through solutions containing 70 percent, 80 percent, 95 percent, absolute ethyl alcohol, and, finally, a mixture of ether and absolute ethyl alcohol in equal parts. The tissue remains in each solution for eight to 16 hours. The embedding medium of choice was celloidin. Paraffin proved to be less satisfactory.

Although there are a great number of fluorochromes on the market, in the routine hematoxylin-eosin stain, eosin provides an excellent fluorochrome and was exclusively used in the present study.

Results

When illuminated by light with inclined incidence, the eosinophilic stained tissue fluoresces. The fluorescence is generally of green color and is particularly marked in all those tissue components which are known for their high content of mucopolysaccharides. The most colorful changes were found in Bowman's and Descemet's membranes, the corneal stroma, the lens capsule, the elastic elements in the blood vessels, the connective tissue of the lid adnexa, and the orbit. To a lesser degree, they were also apparent in

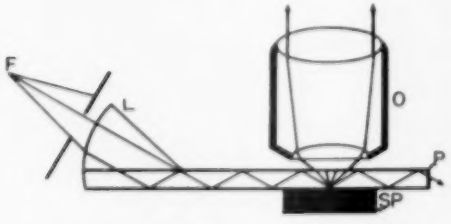

Fig. 3 (Cibis). Schematic diagram of arrangement providing oblique reflected illumination of an opaque specimen attached by contact fluid to the inferior surface of the glass plate (P).

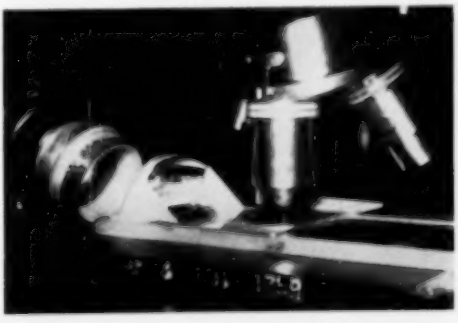

Fig. 4 (Cibis). Photograph of arrangement used in this study. Plane-parallel glass plate placed at microscope stage. Substage illumination used for transillumination, Spencer microlamp (Universal) providing the light for unilateral dark-field illumination. All filters removed.

Fig. 5 (Cibis). (Left) Photomicrograph of normal lens, transillumination. Papilloma of the cornea. (Right) The same lens section with discontinuity zones elicited by light of oblique incidence. Patient, aged 71 years. (Formalin 10 percent, hematoxylin-eosin, ×18.)

the sclera and the meningeal sheaths and septa of the optic nerve.

The protein of the lens fibers binds eosin more than any other tissue components in the eye but does not display green fluorescence under normal circumstances. The histologic section of the normal-appearing lens in Figure 5 is characterized by a uniformly pink tint in transmitted light. In light of inclined incidence the same lens displays a colorful pattern of "histologic zones of discontinuity" (figs. 5 to 9). The principal color characteristics and measurements of these zones in a median section are given in Table 1. A most striking feature revealed by this technique is the presence of relatively coarse granular particles of orange fluorescence in a less granular or almost homogenous groundsubstance as shown in Figure 9. The density of these granules and their size seem to constitute important elements of the histologic zonification particularly in the various portions of the fetal and the adult nuclei (figs. 6, 8, and 9).

The histologic zones are not boundaries between areas of different refractive indices but rather shells of lens fiber components which are distinguished from each other by a variety of characteristics based on color appearance, fluorescence, iridescence, graini-

TABLE 1

AXIAL THICKNESS OF THE HISTOLOGIC ZONES OF DISCONTINUITY OF THE LENS SHOWN IN FIGURES 5–9 (SEE DIAGRAM OF FIGURE 10)

	Designation of Zones	Axial Thickness in mm.	Color Appearance
1	Anterior capsule	0.04	Bright green
2	Epithelial layer	0.01	Violet
3	Anterior stripe of disjunction (subepithelial zone)	0.03	Olive-green
4	Anterior cortex	0.21	White-green
5	Anterior adult nucleus	0.65	Orange-red
6	Anterior dark interzonal stripe	0.01	Dark orange
7	Anterior granular shell of outer fetal nucleus	0.02	Bright orange
8	Anterior dark intrazonal stripe	0.02	Dark violet
9	Anterior outer fetal nucleus	0.15	Orange-violet
10	Anterior granular shell of inner fetal nucleus	0.15	Bright orange
11	Cleft of granular shell (10)		Dark Violet
12	Inner fetal nucleus	1.00	Orange
13	Posterior granular shell of inner fetal nucleus	0.06	Bright orange
14	Posterior inner dark intrazonal stripe	0.02	Dark violet
15	Posterior outer fetal nucleus	0.13	Orange-violet
16	Posterior granular shell of outer fetal nucleus	0.02	Bright orange
17	Posterior dark interzonal stripe	0.02	Dark violet
18	Posterior adult nucleus	0.80	Orange
19	Posterior cortex	0.25	White-green
20	Posterior stripe of disjunction (subcapsular zone)	0.03	Olive-green
21	Posterior capsule	0.01	Bright green

Fig. 6 (Cibis). The same lens as in Figure 5. Zonular structure of axial portion of fetal nuclei. Note graininess of inner fetal nucleus as compared to outer fetal nucleus which shows a dark intrazonular stripe, ×105.

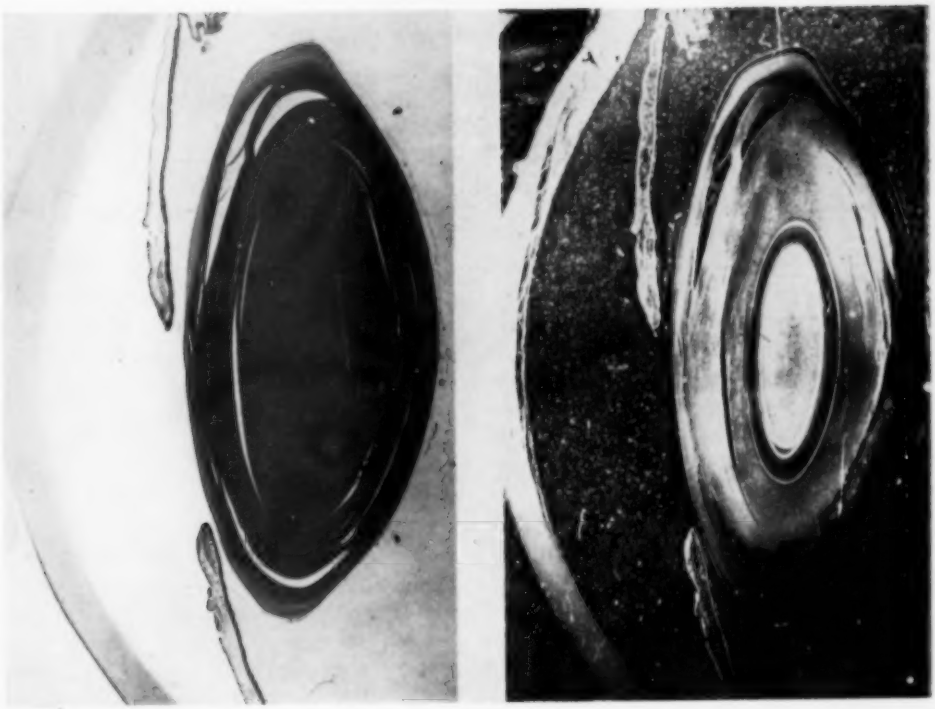

Fig. 5

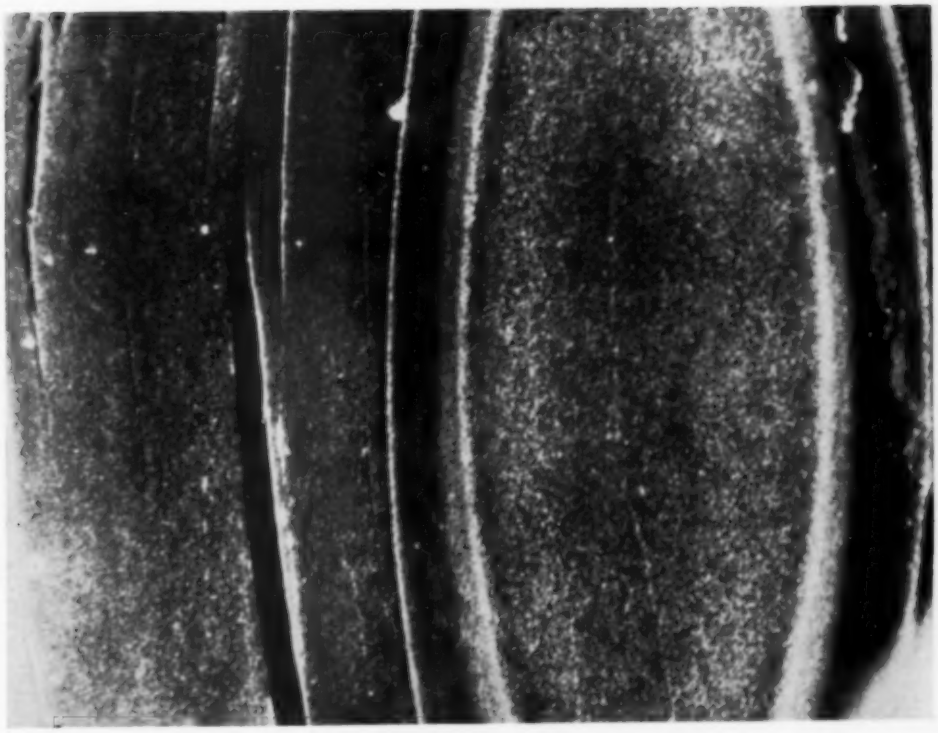

Fig. 6

Fig. 7 (Cibis). The same as Figure 5 (left), portion of equator and lower half of fetal nuclei. Note uniformity of lens substance under condition of transillumination. ×98.

ness, and density of the granular elements which in hematoxylin-eosin stained slides appear in brilliant orange, orange-red, violet, or green colors.

The adult nucleus and outer fetal nucleus (figs. 8 and 9) are separated from each other by a narrow zone of less granularity which may be designated "dark interzonal stripe." In some areas of Figures 9 and 10 the optical emptiness of this stripe is apparent.

The inner fetal nucleus is surrounded by a relatively broad shell consisting of orange granules of fine graininess and great density. This granular band is actually the result of a more or less complete fusion of two granular zones as indicated by the cleft at the equator of the inner fetal nucleus (figs. 9 and 10).

It must be emphasized that all of the above findings are manifestations of secondary fluorochromy. The demonstration of primary fluorochromy requires different techniques. There is good evidence that primary fluorochromes responsive to visible light are present in the ocular tissues. Figure 11 shows a histologic section of the eye of an angel fish whose lens contains a yellowish-brown pigment in the cortex. This pigment fluoresces as a double ring, one in green, the other in a pinkish color, under dark-field illumination. This section* has been fixed with bromeformol and stained with silver carbonate after Scharenberg's[11] modification of the method of del Rio Hortega. It is most likely that primary fluorochromes are largely lost in the routine procedures used in preparation of histologic sections of the eye.

Secondary fluorescence seems to depend greatly on the condition of the proteins and protein derivatives to which the fluorochrome is bound. The normal lens protein stained by eosin fluoresces orange-red in the nuclear zones, pale green in the cortex, and dark olive-green in the zones of disjunction. Under pathologic conditions, however, the entire lens exhibits brilliant green fluorescence. The intensity of the green fluorescence decreases and assumes a dull olive-green, green-blue, or green-gray appearance the more the cataractous changes advance toward the stage of hypermaturity.

The most intensive fluorescence of green or yellow-green color observed so far has been exhibited by the lens capsule, the zonula fibers, and the vitreous body. Thin sections of the vitreous body which do not show up very well under transmitted light are readily visualized when dark-field illumination is applied (fig. 12).

The effect of dark-field illumination on the nuclei of epithelial and endothelial cells is less revealing. Normal nuclei appear faintly violet. In many instances, they display a brilliant fluorescence of yellow color. This effect is particularly outspoken in nuclei of fibroblasts, certain nuclei of the brain, spinal cord, and others.

The dark-field illumination technique applied in this study seems to be also of great value for the recognition and differentiation of pigment granules. Those derived from chromatophores are distinguished by a silvery iridescence, whereas those pertaining

* Courtesy of Dr. Reimer Wolter, University of Michigan, Ann Arbor, Michigan.

Fig. 8 (Cibis). The same as Figure 7 but illuminated with light of inclined incidence. Note cleft at equator of inner fetal nucleus and fusion of the granular shells surrounding it. Dark intrazonular interval within outer fetal nucleus, smaller interval separating the outer fetal from the adult nucleus, ×98.

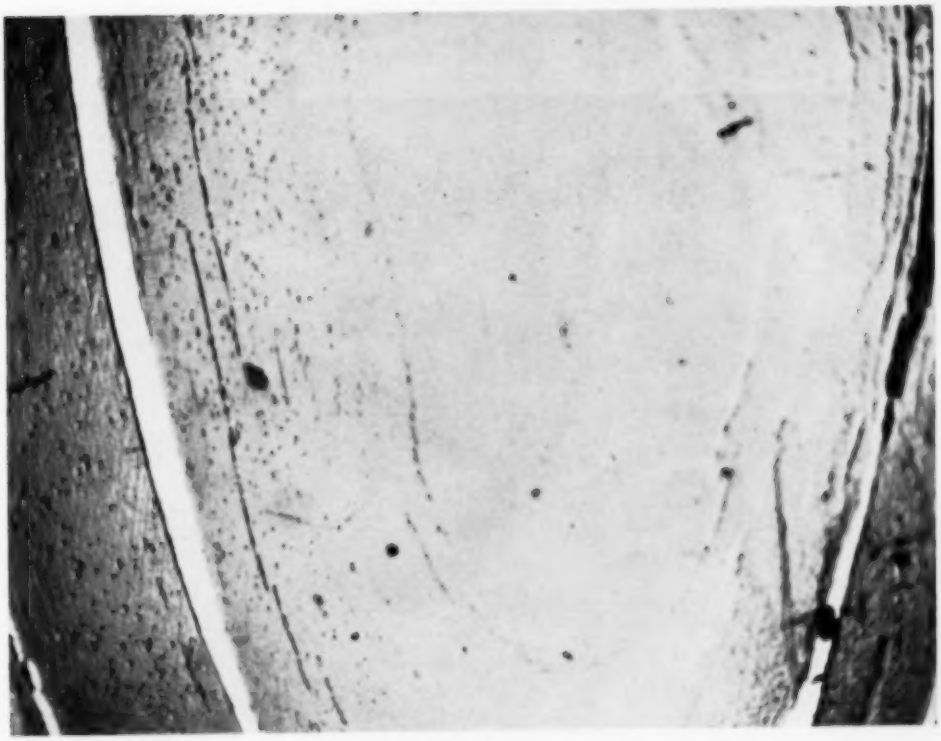

Fig. 7

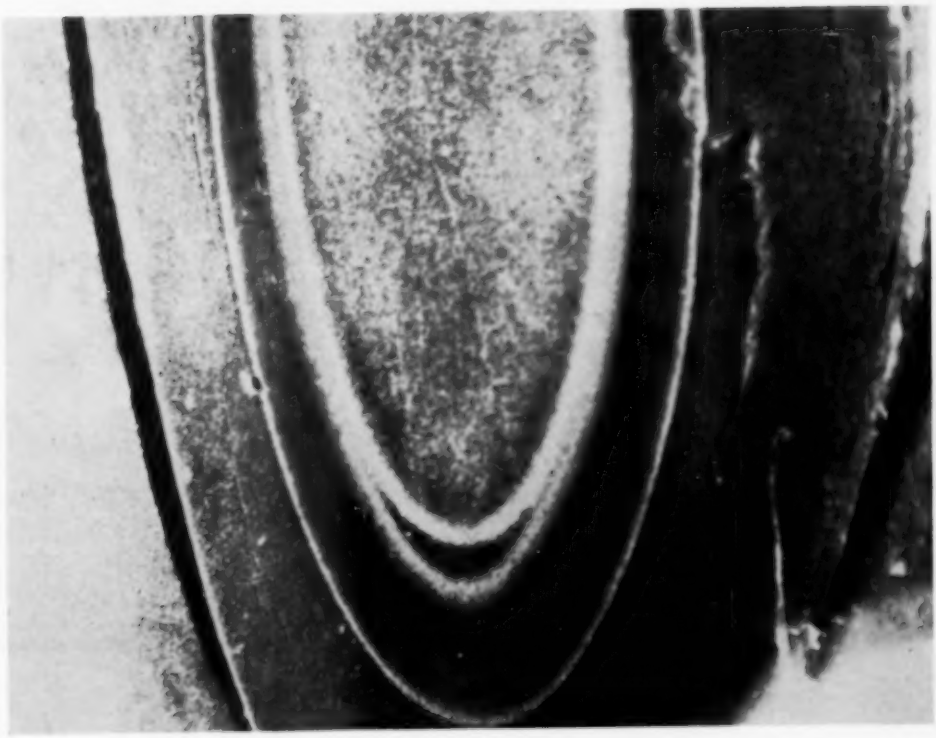

Fig. 8

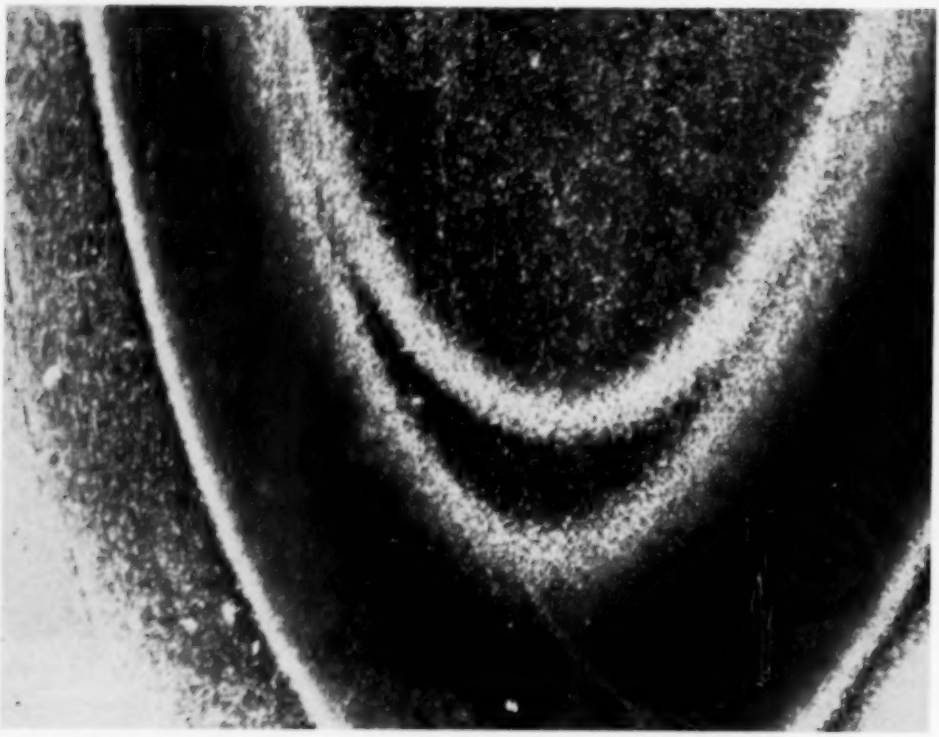

Fig. 9 (Cibis). The same as Figure 8, showing the distribution of the granules within and around the lower pole of the inner fetal nucleus. The granules are of orange-red color, ×240.

to or derived from the pigment epithelial cells, ciliary body, iris, or the pigment layer of the retina appear as purple or reddish brown particles. This is of certain significance for the chromatophores in the meshwork of the ciliary muscles or vascular layers where they can be discovered by transillumination only with difficulty but are easily revealed by light of inclined incidence.

The muscular elements of the ciliary body show up as a violet meshwork under oblique illumination and can easily be distinguished from the connective tissue which exhibits a brilliant green fluorescence, particularly in cases of hyaline degeneration of the vascular layer. It is interesting that the trabecular meshwork resembles the ciliary muscles in its dull violet appearance in contrast to the bright green fluorescence of the scleral spur and the substantia propria of the cornea. Work is in progress to determine the value of this method in the study of the normal histology of the eye and the effects of pathologic processes.

Discussion

The principle of internal scatter as employed in this study is an ancient one and well known to ophthalmologists. The same principle has found wide use in edge-light illumination, in advertising devices, and in many other commercial applications. Although Palmer[8] obtained a patent in 1886 for beveled tissue slides on which sections of specimens could be mounted for the microscopic study in scattered light, the method has been used very little.

The present technique has the advantage

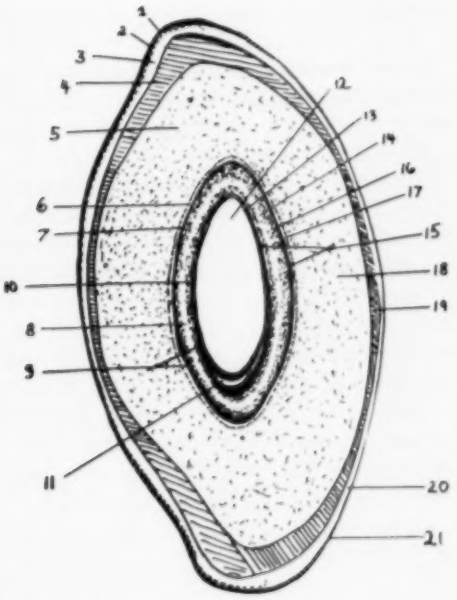

Fig. 10 (Cibis). Diagram of zones of discontinuity as revealed in lens of Figure 5. Designations and measurements given in Table 1.

of a light of inclined incidence from a strong source which can be focused on the microscopic field resulting in dark-field illumination with high contrasts. Another advantage is that dark-field and bright-field illumination are rapidly interchangeable.

The observations made with the technique described above are interesting from several aspects:

First, they prove that "uniformity" of tissue sections observed under the conventional technique of transillumination does not imply uniformity from a physical, chemical, structural, or architectural view point.

Secondly, the observation of zones of discontinuity histologically opens new avenues for the study of the nature of these zones.

Vogt[2] offered suggestions in his textbook and atlas of slitlamp biomicroscopy concerning this problem. He studied the lens fiber structures of sections which had been prepared following the technique recommended by Rable[8] and found variation in the thickness of the lens fibers of various zones.

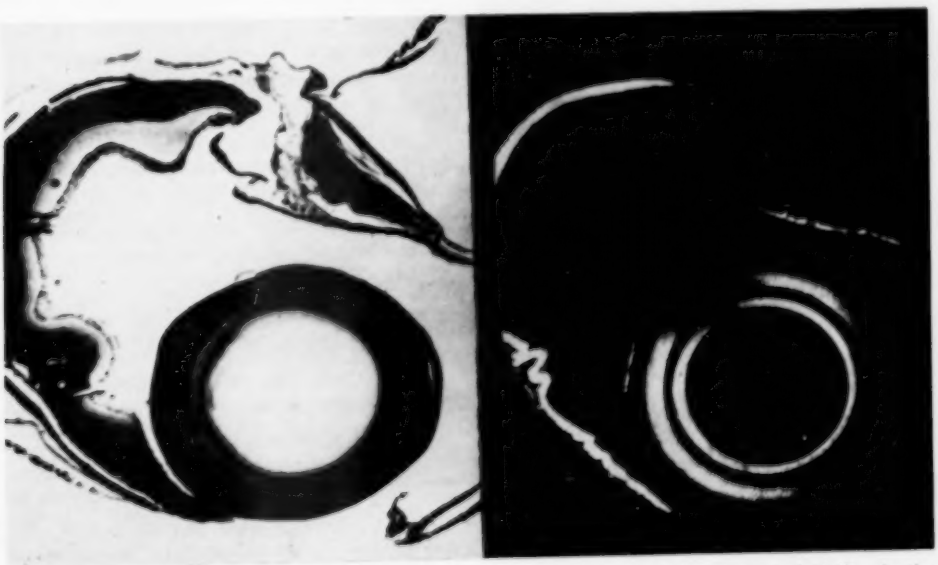

Fig. 11 (Cibis). Lens of angel fish as seen under transillumination (left) and dark-field illumination (right picture). Outer ring fluorescent violet-brown, inner ring fluorescent blue-green. Bromo-formalin fixation, del Rio Hortega stain.

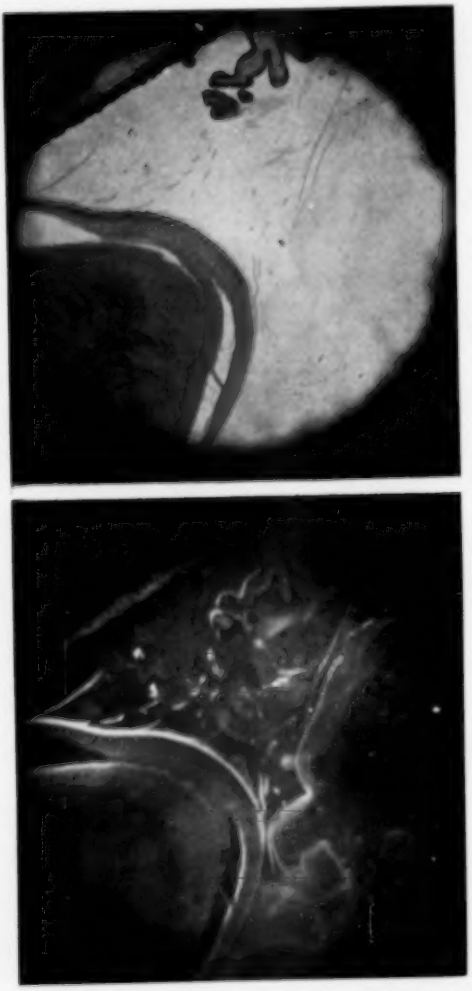

Fig. 12 (Cibis). (Above) Portion of lens with faintly visible fragments of zonula fibers, diffuse illumination by transmitted light. (Below) The same section in dark-field illumination. Note distinctiveness of lens capsule, zonula fibers, and vitreous body (in the microscope appearing with brilliant green fluorescence). (Formalin 10 percent, hematoxylin-eosin, ×18.)

These observations led him to believe that variation of lens fiber thickness represented the anatomic substrate of the biomicroscopic zones of discontinuity. He rejected Goldmann's suggestion that the discontinuity zones are the result of growth inhibition due to traumatic influences during the development of the lens.

Rable[8] himself found the thickness of the lens fibers extremely varying in mammalian lenses, particularly in primates, and emphasized that he could not discover the substrate of any special "zones." Rable's solution as well as Zenker's fixative employ mercury bichloride which bring out the structure of the individual lens fiber very nicely but fail to elucidate zonular discontinuities. Variation in the thickness of the lens fibers was particularly noted in eyes which had been subjected to X radiation or other trauma (sodium iodo-acetate).[10] However, manifestations of zones of discontinuity have never been seen in such cases although one should expect them when Vogt's concept is true.

It had been realized for a long time that in the preparation of eye sections artefacts are easily introduced which may mask in vivo lens changes. Reese and Wadsworth[9] considered the interpretation of lens changes by means of microscopic sections as notoriously treacherous. Not infrequently it was impossible to differentiate pathologic changes from normal (Berliner).[6] Consequently, many ophthalmologists regarded the histopathology of the lens of little value and permitted their technicians to remove the hardened lens prior to cutting to avoid damage to the microtome knife. Much work is needed to improve present histologic techniques in preparing lens sections.

Summary

A microscopic illumination technique is described which permits simultaneous or alternating employment of bright-field and dark-field illumination.

Application of the new technique in routine microscopy of ocular and other tissue sections stained with hematoxylin and eosin led to a series of observations which are of interest to the ophthalmologist, histologist, pathologist, and biologist.

When applied to ocular tissues the tech-

nique of alternating bright- and dark-field illumination revealed:

1. "Histologic zones of discontinuity" in the lens comparable to the "biomicroscopic zones of discontinuity" of Vogt.

2. Differential manifestations of secondary fluorochromy induced by visible light in eosin-stained proteins and polysaccharides of normal and pathologic tissues.

3. Differential effects of primary fluorochromy induced by visible light in sections of a fish lens stained by the silver technique of del Rio Hortega after fixation in bromoformol.

The illumination device, the technique of its application, and pertinent observations are illustrated by a series of photographs. The potentialities of the new technique are discussed.

640 South Kinghighway (10).

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Discussion

Mr. Lee Allen (Iowa City, Iowa): Several months ago Dr. Cibis demonstrated his new illuminating device for Dr. Burian. He was impressed by the zones of discontinuity in the lens and the emphasized relationships elsewhere in the eye, but took special note of those at the chamber angle because of possible value in our continuing study of that region. Dr. Cibis was good enough to furnish us with specifications of his device and instructions for its use. Since then we have observed most of the effects Dr. Cibis has described.

His excellent photographs and descriptions of findings such as the zones in the lens require no comment. However, we would like to ask the author whether he has correlated these latter zones with those seen with the slitlamp biomicroscope.

Also we would like Dr. Cibis to explain further how eosin can appear red in one part, yellow in another, and blue-green in the balance of the same general tissue such as the lens cortex. If graininess and density are the key, is it because eosin is trichromic but that the physical and chemical properties of the tissues can alter the filtering characteristics of the dye? It seems that the potential value of the method may depend in part upon a clear understanding of these effects.

This method has not helped us greatly, as yet, in the study of the chamber angle, although some of the developing clefts in embryonic eyes show very prominently in the dark field.

The first slide is of the angle of an adult eye fixed in formalin, stained with hematoxylin-eosin, and illuminated with Dr. Cibis' method. The cornea and sclera appear green, the entire meshwork violet, some of the connective tissue blue, and pigment orange. Incidentally we were slightly taken aback because we had expected certain fibers nearest Schlemm's canal to have some characteristics of the cornea and sclera.

The second slide shows, in the upper picture, the chamber angle of a dog's eye fixed in Zenker's, stained with hematoxylin-eosin, and seen in bright-field illumination. Most of the tissue appears pink except for the darker brown pigment. The lower picture is of the same slide illuminated by Dr. Cibis' method. The corneal epithelium is violet, the stroma greenish blue, the endothelium violet, the sclera light greenish blue, the trabecular meshwork violet, pectinate ligament strands light cream, iris muscles gray, and pigment yellow to orange.

Dr. Cibis' method is a special type of dark-field illumination. In it, the light approaches from behind and from one side only, and the light is inclined at a more acute angle than with the standard dark field condenser. His plate glass and prism arrangement for obtaining just the right oblique, rear

illumination, closely approach the requirements for reproducible effect desired of a scientific procedure.

We have been able to achieve the same results, except for ease in changing to bright-field illumination and for control, by several other means. For the laboratory, a dissecting microscope may be used with a pocket mirror placed in a horizontal plane about one inch below the glass stage and half-way between the microscope and the lamp. The lamp and mirror may be manipulated to achieve the best dark field and color effects.

The third slide shows how the slitlamp biomicroscope may be used by opening the angle between the slit beam and the microscope and holding the histologic slide at the point of common focus. The slit aperture should be wide open. The angle of the beam may be varied through an arc of 180 degrees, thus allowing a wide variation of effects. To approximate Dr. Cibis' results closely, the angle of the illumination should be very oblique.

The fourth slide demonstrates the appearances of various eye sections as they were illuminated with the beam of the slitlamp. These photographs were taken with a view camera rather than through the microscope of the slitlamp. All the sections were Zenker fixed and stained with hematoxylin-eosin. The circle at upper left is a cataractous lens in bright field; beside it is the same lens in oblique illumination. At the upper right is an anterior segment showing especially the zones in the lens. At the lower left is the chamber angle region of a dog's eye. The two pictures at the lower right are two exposures of the optic nerve.

In closing, we want to emphasize that the simplicity by which one may shift from oblique illumination to bright field for careful correlation of the two effects, makes Dr. Cibis' device far superior to the two we have mentioned. The latter may serve largely to help demonstrate the basic nature of the general method.

We wish to thank Dr. Cibis for the opportunity of using his device and the privilege of discussing his paper. Finally, we wish to congratulate him upon the ingenuity of his device and upon the excellent observations he is making with it.

Dr. Hermann M. Burian (Iowa City, Iowa): I want to report very briefly an observation which I made and which I thought rather interesting. I happened to look at a slide of a 110-mm. embryo with oblique illumination on the slitlamp and noted, to my surprise, while looking at the cornea, that there were two parts very clearly defined, an anterior green and a posterior yellowish-golden part. One could clearly follow the yellow part into the mesodermal area and the green part into the ectodermal area.

So, by Dr. Cibis's method an embryonic cornea stained with eosin, which looked perfectly homogeneous in transmitted light, was clearly differentiated into its two embryologic layers.

I was fascinated by this and I mention it here to add my word of praise for Dr. Cibis's method and to point out its possible value for embryologic studies.

Dr. David Cogan (Boston): Dr. Cibis's slides speak for themselves, both as to esthetic and scientific interest. I would like to ask Dr. Cibis a question that is not strictly germane to his paper. How does he account for the zones of discontinuity in the lens?

The lens is a continuously growing organ and, as far as I know, it does not have any cyclic increases or decreases in growth. It is a continuous phenomenon. These zones, which we have seen so dramatically, and which are evident by other means to some extent, have always been an enigma to me. I wonder if Dr. Cibis would care to comment on them.

Dr. J. Reimer Wolter (Ann Arbor, Michigan): This new illumination technique, the use of which enables Dr. Cibis to demonstrate such revealing facts in preparations of normal and pathologic eyes, doubtless is a completely new and very important invention. The simple but ingenious instrument seems technically somewhat similar to a dark-field illumination attachment. But its effect is much more like that of a phase-contrast microscope. Both instruments, the phase-contrast microscope and the Cibis-condensor used on a common microscope, make it possible to visualize structures of the tissue by their physicochemical differences.

The present study of Dr. Cibis indicates that this illumination technique is superior to phase-contrast microscopy in some fields of eye pathology. I have no doubt that the Cibis-condensor will open a large field of new investigations and greatly enlarge the spectrum of our histologic methods.

Dr. Paul A. Cibis (closing): First, may I answer Dr. Cogan's question. The granularity or graininess as shown in the histologic sections, of course, is not present in vivo. It presents precipitations produced by the formalin. I believe, therefore, that they indicate some differences in the chemistry which, from a physical viewpoint, may indicate differences in the refractive index and may show up as such by observation with the slitlamp.

These discontinuity zones (and this is just speculation) can be produced either by differences in the refractive index or by the Tyndall phenomenon. If there are opacities or surface irregularities of the structural elements, they may produce diffuse scatter and show up that way. I don't believe there is a final answer that can be offered.

With regard to Dr. Allen's question why the eosin fluoresces in one case and why it does not in another, I must say again that I don't know. I believe, from the observations, that fluorescence indicates pathologic conditions. It must have something to do with the breakdown of the protein to which the eosin is bound.

A QUANTITATIVE ANALYSIS OF THE HORIZONTAL MOVEMENTS OF THE EYES IN THE EXPERIMENT OF JOHANNES MUELLER*

I. Method and Results

Mathew Alpern, Ph.D., and Paul Ellen, Ph.D.
Ann Arbor, Michigan

Introduction

The manner in which variation in the dioptric power of each eye in far to near focus (that is, accommodation) is related to the relative position of the lines of sight of the two eyes (that is, convergence) has been subject to controversy for many years. Johannes Mueller was the first (1826) to provide objective evidence that these two functions are related. Mueller noted that if one eye was occluded and the other eye changed fixation from a far to a near object along the same line of sight, the occluded eye moved nasalward.[1]

Although considerable information regarding the relation of these two functions has been accumulated in the century and a quarter since these experiments were first described, there remains even today no uniformity of opinion as to the way in which Mueller's original results can best be explained.

Even with the definitive photographic data of Allen[2-4] obtained at two different accommodative stimulus levels, there is a dearth of information as to the time characteristic patterns of the change in accommodative convergence following the onset of far to near change in focus, and the way these patterns change with a change in the stimulus to accommodation. It seemed important, therefore, in order to gain a better understanding of Mueller's experiment in particular, and of the interrelation of accommodation and accommodative convergence in general, to repeat the original experiment at a variety of different accommodation stimulus levels in such a way that a quantitative analysis of the horizontal eye movements during the change in fixation from far to near could be formulated. It is possible that such an analysis may eventually form the point of departure for a more adequate theoretical formulation of accommodation and convergence data.

Method

The experiment used the ocular rotational potential (ORP) to study the change in position of the eyeball during Mueller's experiment. The details of this method have been described elsewhere[5] and consequently only a brief outline of the procedure will be presented. Figure 1 illustrates a schematic view of the apparatus. The subject sat in a well-lighted but electrically shielded room. Grass-electroencephalograph silver disk electrodes and Offner electrode paste were placed on the face in such a way that differences in potential between the electrodes at the inner and outer canthus of each eye could be amplified and recorded in separate channels of an Offner D.C. amplifier and dynograph. A third channel of the dynograph was used to record the stimulus onset using either a microphone or a push-button as a signal marker. Electrodes were aligned also vertically above and below each eye and these electrodes as well as an electrode from the right ear were led to the ground.

The subject was seated comfortably in a chair and her head was placed in a chin rest. The left eye was occluded and the right eye fixated the mirror image of vertical plumb line at a distance of 3.4 meters from the spectacle plane. A piece of black surgical thread was mounted horizontally on an

* From the Vision Research Laboratories and the Department of Ophthalmology, The University of Michigan. Supported by Contract DA-36-039-SC-52634 between The University of Michigan and the U. S. Army Signal Corps.

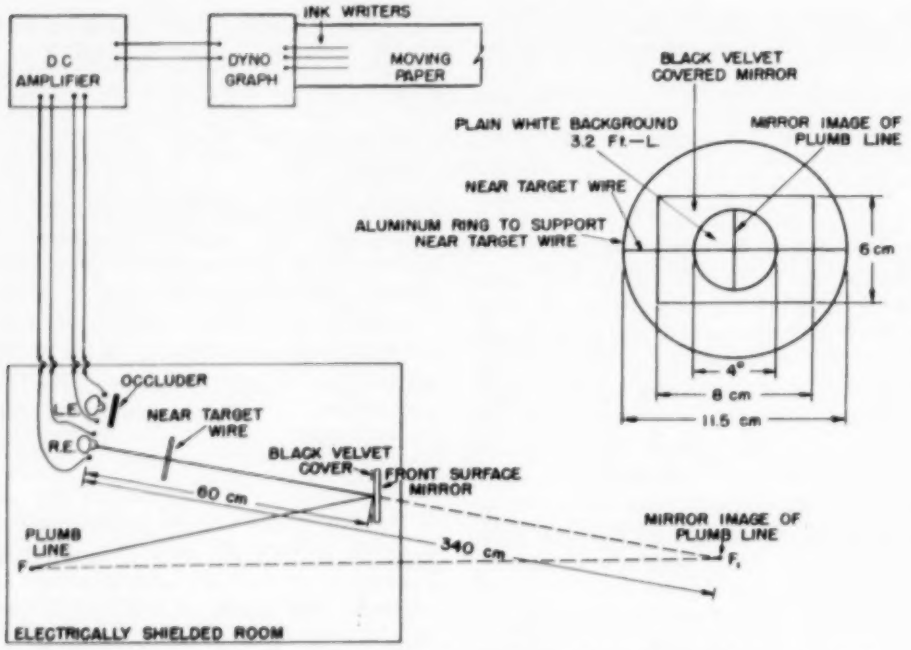

Fig. 1 (Alpern and Ellen). Schematic view of the apparatus and arrangement of test target field.

aluminum ring (11.5 cm. in diameter) supported by a ring stand at various near distances to the eyes. At a signal from the experimenter, the subject shifted her focus from the far (vertical) line to the near (horizontal) line. The subject was instructed to maintain fixation at all times on the point of intersection of the vertical and horizontal lines and, on the signal, change merely the line upon which she focused. After several practice sessions, the subject reported no difficulty in changing focus without altering fixation.

The parameter in these experiments was the separation of the far and near targets. The position of the far target remained fixed at 3.4 meters but that of the near target was varied in such a way that the difference in the stimulus to accommodation at the subject's spectacle plane provided by the separation of the far and near targets was either 7D., 6D., 5D., 4D., 3D., 2D., or 1.4D. Measurements at a given separation constituted one experimental series. Usually a separate day was required for each series, but occasionally two series were completed during a single experimental session with suitable rest periods intervening. The experimental targets were seen against a four-degree circular white background whose luminance, as measured with a Macbeth Illuminometer, was 3.2 foot-lamberts. The immediate surround was a black rectangular velvet cloth covering the surface of the mirror which reflected the image of the plumb line.

In order to convert the ocular rotational potential to degrees of eye movement, it was necessary to obtain a calibration curve at each experimental session. This was done by having the subject shift her gaze from one point in the field to a second point in a known amount of saccadic movement. The amplitude of the ocular rotational potential re-

sponse was obtained for various sizes of such movements and a calibration curve showing the size of the voltage change for each movement was plotted. These curves proved to be linear within the range of movements studied (less than 20 degrees) and, for this subject at least, were remarkably similar from one experimental session (that is, one day) to the next.

Results

Typical results of this experiment are illustrated in Figure 2 (change from far to near) and Figure 3 (change from near to far). These figures illustrate the form in which the raw data were obtained and considerable correction (for curve in the arc of the pen and for calibration) is required before such data can be transformed into a linear scale of movement of the eyes. Nevertheless, even these raw data emphasize the invariable finding in this experiment, that for this subject all eye movements are confined to the occluded eye.

What then is the nature of the movement

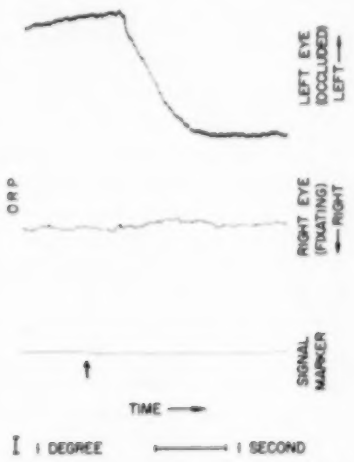

Fig. 2 (Alpern and Ellen). Ocular rotational potential (raw record) of the movements of the eyes in the experiment. At the signal the observer changed focus from the far (3.4 meter) to the near (0.189 meter) target. The difference in the stimulus to accommodation was five diopters.

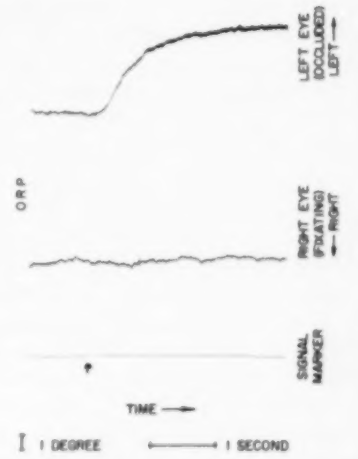

Fig. 3 (Alpern and Ellen). Ocular rotational potential (raw record) of the movements of the eyes in the experiment. At the signal the observer changed focus from the near (0.189 meter) to the far (3.4 meter) target. The difference in the stimulus to accommodation was five diopters.

of the occluded eye to the change in the stimulus to accommodation? Figure 4 illustrates (in the top curve) a more detailed analysis of a typical set of such data. This curve is the mean of the individual determinations of the movement from the far (3.4 meter) to the near (0.159 meter) target, the difference in the stimulus to accommodation being six diopters. The smooth curve has been fit by eye to show the general trend. In this figure the time interval from the onset of the movement is represented along the abscissa. It should be emphasized that for averaging the individual curves it is important that the zero abscissa value be taken as the time of the onset of the movement and *not* as the time of the onset of the stimulus, if the averaged curve is to have a form which is representative of the individual curves. This is true because successive measurements have widely differing reaction times. (If the zero time is taken as the onset of the stimulus then the average curve of two curves with markedly different reaction times will have a form, particularly at the lower

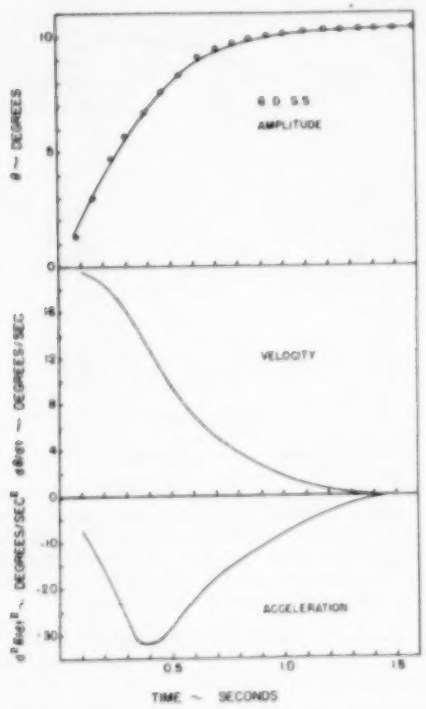

Fig. 4 (Alpern and Ellen). (a) The top curve: the amplitude of the movement of the occluded eye in degrees as a function of time (in seconds) following the onset of the movement. The data are the averages of 10 individual measurements after appropriate corrections have been made. The smooth curve has been fit by eye. The first 50 msec. cannot be measured precisely with pen oscillograph. (b) Middle graph: the variation in instantaneous velocity as a function of time following onset of the movement. The smooth curve has been obtained graphically from the smooth curve in the top figure. curve is obtained by differentiation of the smooth (c) Lower graph: the acceleration as a function of time following the onset of the movement. The curve is obtained by differentiation of the smooth curve in the middle graph. The separation of the far and near targets in this experiment was such that the change in focus was from far-to-near and the difference in the stimulus to accommodation was six diopters.

part of the curve, which is strikingly different from either of the two individual curves.)*

* Dr. W. M. Kincaid of the Vision Research Laboratories brought this artefact in data analysis to our attention.

In the top graph of Figure 4 the curve is continuously smooth reaching a maximum value without any overshoot. That is, the movement appears to be critically damped. This has proved to be the case with all of these experiments with this subject. Prolonging the measurement as long as seven or eight seconds showed that once the maximum value was reached no further change in the record of eye position was apparent other than what can be attributed to background "noise" or "drift" in the electrical system. Consequently the curve in Figure 4 has been studied only up to and immediately beyond the point where the maximum value has been definitely reached. By definition, consequently, the *duration* (t) of the movement is the value of the time interval between the onset of the movement and the moment when the maximum amplitude is first achieved.

The data in the top curve of Figure 4 show the averaged results of 10 measurements of the far to near shift of gaze. Figure 5 gives some idea of the most extreme case of vari-

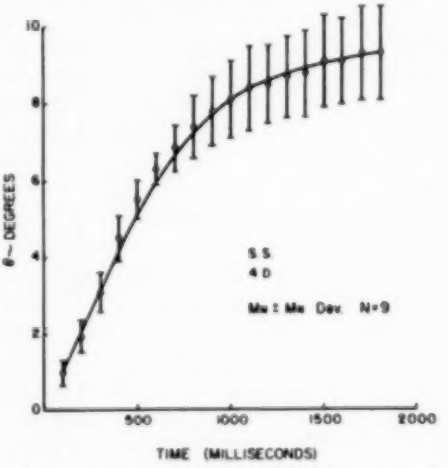

Fig. 5 (Alpern and Ellen). Variability of successive measurements in the shift from far-to-near. The ordinates in the figure show the amplitude of the movement in degrees, while the abscissas show the time following the onset of the movement in milliseconds. The target separation was such that the difference in the stimulus was four diopters. The scatter of these data represent the most extreme found in this experiment.

ability obtained. Although a considerable degree of scatter is present, the curves all showed the same tendencies; what variability occurs in the amplitude of the movement is probably related at least in part to variations in the accommodative response to the same stimulus to accommodation in successive determinations. Each curve, in general, also demonstrated a smooth continuous function; whatever discontinuities occurred can be attributed to background "noise" of the subject to "drift" or to the inability to measure the raw records with a sufficiently fine scale to pick up the slowly changing difference. The averaging process tends to smooth out irregularities arising from "noise," "drift," or scaling difficulties.

The middle graph in Figure 4 shows the variation of the instantaneous velocity of the movement as a function of time following the onset of the movement. The smooth curve has been obtained by graphical differentiation of the smooth curve in the top graph in this figure and is typical of the smooth velocity curves obtained in these experiments. The velocity of the movement was invariably largest in the early part of the movement and could be as high as 20 degrees/second. Typically this maximum velocity was maintained for some milliseconds before the velocity began to drop off and only gradually reached zero as the movement was completed. In subsequent discussion, consideration will be given to the *average* as distinct from the *maximum* velocity. The average velocity is defined as the total amplitude of the movement divided by its duration. In general, the average velocity varies with the parameter in essentially the same way as did the maximum velocity but the absolute values of the latter were, of course, larger (by a factor of approximately 2.5) than the former.

The lowest graph in Figure 4 shows the variation in instantaneous acceleration of the movement as a function of the time following onset of the movement. These values were obtained by graphic differentiation of the smoothed velocity curve of the middle graph in Figure 6. The acceleration decreases to a high negative value around 400 milliseconds and then gradually returns to zero, the duration of the former period being much shorter than the duration of the latter.

Discussion

The absence of any large discontinuities in these curves suggests that the far-to near change in focus occurs as a smooth continuous inward rotation of the occluded eye. This fact is important since in these experiments two factors operate together, each of which may influence, independently, the amount of inward rotation of the eye.[6] These are: (a) the degree the test target is out of focus and (b) the near position of the test target.

If, as has been suggested,[7] the influence of each of these factors may occur at the output end of the system, that is, that one may differentiate between accommodative convergence and proximal convergence, then it might be expected that two distinctly different types of movements might be distinguished, with respect to time characteristics. This should be apparent in the eye movement curves as sharp discontinuities which would presumably become more pronounced as the near target approaches the eye.[6,7] The absence of such discontinuities, consequently, suggests that in the present experiment either (a) the proximity factor made no major contribution to the position of the eyes during the movement (as may occur on occasion) or (b) the time characteristics of the two types of movements are identical. With the data at hand it is not possible to decide which of these conclusions is justified.

One important finding of these measurements is that, for our subject at least, only the occluded eye moves. The surprising fact is not that the movement of the fixating eye is damped but the way in which it is damped. According to the usual textbook description,[8] the fixating eye should make a saccadic

movement to the right (in Figure 2) and then the convergence movement should be completed as a yoked binocular act—the amplitude of the movements of each eye being identical. Figures 2 and 3 illustrate clearly that this is not the case in Mueller's experiment. However, there is considerable evidence that, at least for some stimulus conditions, the textbook description is reasonably adequate (at least qualitatively).

Figure 6 illustrates an example where this is the case. The experiment is similar to that illustrated in Figure 2, except that fixation is now binocular (the near target is a vertical line which had been carefully aligned *monocularly* on the line of sight of the right eye). Results similar to those illustrated in Figure 6 have also been obtained invariably for this subject when she binocularly fixates a vertical target and a small amount (5 to 15$^{\Delta}$) of prism is suddenly introduced base-in or base-out before the left eye.[9]

The evidence at hand suggests, consequently, that, for this subject at least, the eye

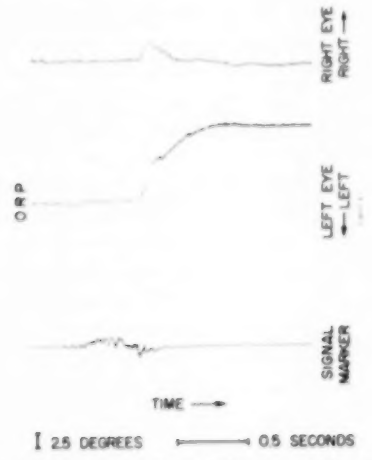

Fig. 6 (Alpern and Ellen). Ocular rotational potential of the movements of the eyes in binocular assymmetrical convergence. The target separation is such that the difference in the stimulus to accommodation was five diopters. The near and far targets were both vertical lines. The near target was carefully aligned monocularly on the line of sight of the right eye before the experiment began.

movements in the experiment of Johannes Mueller (monocular fixation) follow the "principle of least muscular effort," established in another connection by Wundt.[10] In binocular far-to-near focus, however, or in prism vergence testing the eyes move (at least in a qualitative way) according to Hering's law of equal innervation of the ocular muscles of the two eyes.

The way in which Hering's law is believed to operate has changed considerably since it was first formalized. Hering's original notion leads to the speculation that the right eye itself would make "irrelevant" movements in the experiment illustrated in Figure 6. Bielschowsky,[11] on the other hand, emphasized that the eye would not make such irrelevant movements, while none the less reaffirming the essential validity of Hering's law. According to this notion, the yoked movements of that eye would be damped somewhere in the neuromuscular system. Breinin,[12] using electromyographic techniques, has confirmed this notion and offered evidence that the damping of the irrelevant movement does not occur in the eye muscles but presumably only in the nervous system. It should be pointed out, however, that each successive failure to obtain evidence of yoked movement (and the associated reduction in the number of degrees of freedom available for the potential damping mechanisms) must be considered as weakening of the original theoretical position.

Paradoxically, therefore, the results illustrated in Figure 6, which have been independently confirmed by Westheimer[13] using different observers and different techniques, and the results with prism vergence tend to contradict the results of Breinin, while they strongly reaffirm his conclusion that Hering's law remains valid for binocular asymmetric convergence. The discrepancy here is probably one of method. Using needle electrodes it is unlikely that Breinin was able to record simultaneous responses of all of the motor units of the muscles so that it is possible that small movements (such as those

of the right eye in Figure 6) could have occurred without any change in activity being recorded from the needle electrode. On the other hand, the possibility should not be discounted that individual differences in subjects may account for the discrepancies.

These results imply that if Hering's law of equal innervation of the muscles of the two eyes remains valid, then the way in which the yoked movements are damped in binocular asymmetrical convergence is essentially different from the way the yoked movements of the fixating eye are damped in Joannes Mueller's experiment. How can this difference be explained?

One possible explanation arises from the hypothesis of Alpern and Wolter[14] that vergence movements (such as those in Mueller's experiment) are produced by innervation from the autonomic nervous system, while saccadic movements are produced by innervation from the somatic nervous system. In Mueller's experiment, fixation (mediated by the rapid activity of the somatic nervous system) requires the unoccluded eye to remain on target, while the yoked vergence movement would require the eye to move. Because of the difference in velocity characteristics of the two types of movements (and perhaps for other physiologic reasons, as well) the somatic system predominates and the fixating eye fails to move.

In the experiment illustrated in Figure 6, when fixation is now binocular, the conditions are changed. Here the shift from far-to-near is associated with a change in fixation signal (in terms of the retinal feedback from the left eye) as well as an accommodation signal. Now both of these movements are yoked movements which are mutually antagonistic to one another in so far as the right eye is concerned. Nevertheless, a certain amount of movement of the right eye occurs even though its initial and final positions are identical. That is, the fixation movement (mediated by rapidly acting somatic nervous system) forces an irrelevant movement of the right eye, which the slower acting autonomic activity is not able to damp immediately. The magnitude of this "irrelevant" movement is apparently just sufficiently large so that the inward rotation of the eyes associated with the accommodation will be evenly distributed in the two eyes and will return the right eye to the starting point. A similar explanation would account for the "irrelevant" motion of one eye, when a small amount of prism is placed base-in and base-out before the other eye during binocular fixation.[9] It will be interesting to see if such speculations can withstand the test of subsequent experiments.

Summary

Measurements of the way in which the eyes move when fixation is changed from the far to a near target along the same line of sight when the other eye is occluded have been obtained, using the ocular rotational potential. Only the occluded eye moves and some of the characteristics of the movements, such as the instantaneous amplitude, velocity, and acceleration, have been analyzed. Theoretical implications of the data for theories of convergence and for the theory of the law of equal innervation to the muscles of the two eyes are briefly discussed.

University Hospital.

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A QUANTITATIVE ANALYSIS OF THE HORIZONTAL MOVEMENTS OF THE EYES IN THE EXPERIMENT OF JOHANNES MUELLER*

II. Effect of variation in target separation

Mathew Alpern, Ph.D., and Paul Ellen, Ph.D.
Ann Arbor, Michigan

Introduction

In the previous paper in this series[1] the analysis of the horizontal movements of the eyes in the experiment of Johannes Mueller was described in some detail. The present paper is devoted to a description of the way in which the various characteristics of the movement change with a change in the separation of the far and near targets. The apparatus and method have already been described.

Results

Figure 1 shows the averaged curves of amplitude of the movement as a function of time following the onset of the movement for each of the target separations in the far-to-near shifts of gaze. Figure 2 shows the same curves for the near-to-far shift of gaze. In each case the plotted points are the mean values of a fairly large number of repetitions[9-13] of the experiment. In each figure the ordinates show the amplitude of the movement in degrees and the abscissas the time following the onset of the movement in milliseconds; each curve represents the results for a different separation of the target. Some of the curves have been arbitrarily displaced along the time axis to present all the data with a minimum of confusion.

A. Displacement characteristics

Figures 1 and 2 show that there are clear variations in the displacement characteristics as the parameter (target separation) varies. Detailed analysis of these variations is illustrated in Figure 3 which shows the average amplitudes (top set of graphs), the average velocities (middle set of graphs), and the average accelerations as functions of the differences in the stimulus to accommodation (in diopters) provided by the target separations. In each set of graphs the circles

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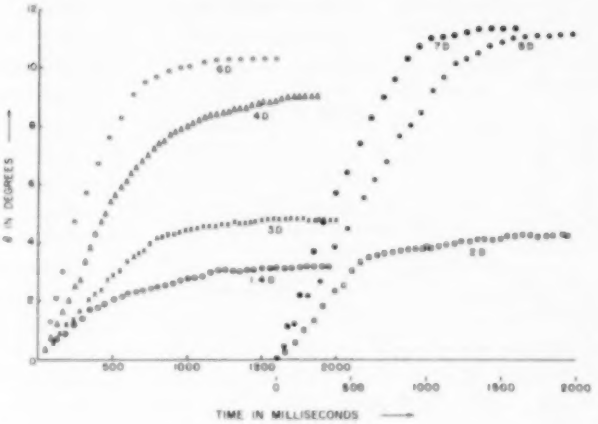

Fig. 1 (Alpern and Ellen). Variation of the movement of the occluded eye with change in the stimulus to accommodation. The data represent the shift from far to near. The curves for change in the stimulus of 7.0D., 5.0D., and 3.0D. have been displaced to the left on the time axis to avoid confusion. The curves are the averages of a number of repetitions (7.0D., N = 15, 6.0D., N = 10, 5.0D., N = 10, 4.0D., N = 9, 3.0D., N = 10, 2.0D., N = 10, 1.4D., N = 10).

represent the shift from far to near and the dots, the shift from near to far. Again, differences between the far-to-near and the near-to-far shifts occur, the latter being in general a smaller, slower movement and having, at least at the smaller target separations, a slightly lower average acceleration. In general, however, the amplitude, average velocity, and average accleration for both types of movements show the same characteristic changes as the target separation increases:

(a) Increasing the target separation increases the amplitudes of these movements. This is what previous experiments would predict,[2] However, at the larger target separations the amplitude appears to reach a maximum, so that a further increase in target separation results in no further increase in the amplitude of the movement and this would not be anticipated by previous experiments,[3] even, if the accommodative response has reached its maximum.

(b) Increasing the target separation is associated with an increase in the average (as well as the maximum velocity) of the movement. The increase of the average velocity reaches a maximum value at about six diopters' difference in the stimulus to accommodation and a further increase in the

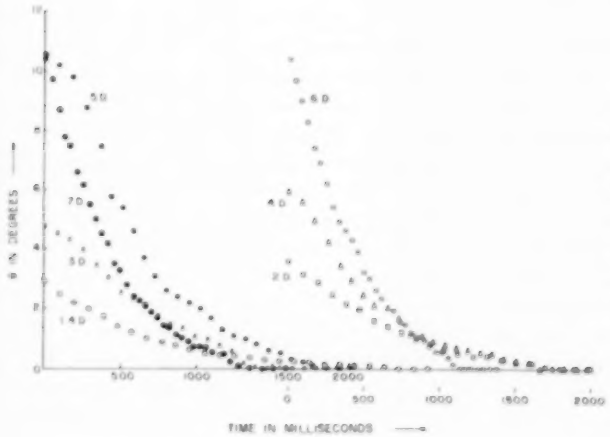

Fig. 2 (Alpern and Ellen). Variation of the movement of the occluded eye with change in the stimulus to accommodation. The data represent the shift from near to far. The curves for change in the stimulus of 6.0D., 4.0D., and 2.0D. are displaced to the left on the time axis to avoid confusion. The curves are the averages of the same number of repetitions as those for the comparable far-to-near curve in Figure 1.

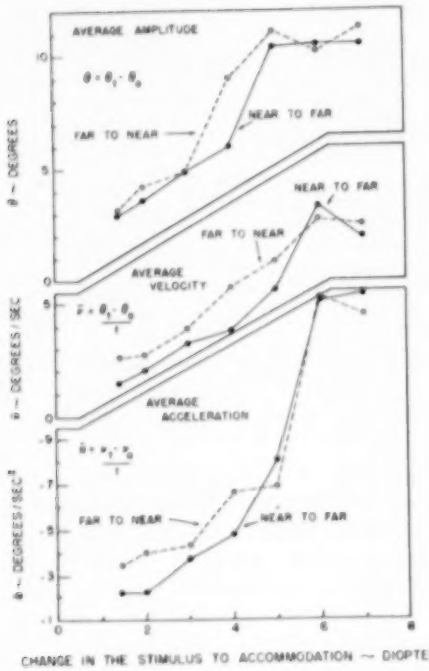

Fig. 3 (Alpern and Ellen). Displacement characteristics as a function of the change in the stimulus to accommodation. Top graph: variation in average amplitude of the movement. Middle graph: variation in the average velocity of the movement. Bottom graph: variation in the average acceleration of the movement. Data obtained from Figure 1 and Figure 2.

target separation results in no further increase in the average velocity of the movement beyond this point. The greater velocity for the shift from far to near than that from near to far was also found for 2.0D. and 4.0D. differences in the stimulus to accommodation by Allen,[4] using photographic recording of the eye movements.

(c) Increasing the target separations was associated with an increase in the average (negative) acceleration of the target up to a difference in the stimulus to accommodation of six diopters. Any further increase in the target separation beyond this point was not associated with a further increase in the average acceleration of the movement.

Figure 3 illustrates an important feature of these movements which has not been generally recognized. At the present time the study of the interrelation of accommodation and accommodative convergence has been largely confined to a study of the amplitudes of the movements. If this criterion alone were applied, then the conclusion would be inferred from Figure 3 that increasing the stimulus separation beyond five diopters would produce no further effect upon the characteristics of the movement. It is only when the velocity and acceleration characteristics are studied that it becomes apparent this is not the case. Such data point up the fact that two movements of equal amplitude are not necessarily similar and emphasize the importance of studying as many features of the movement as possible. It seems important now to know whether such changes in velocity and acceleration without changes in amplitude are associated with changes in the response as well as in the stimulus to accommodation.

B. Time characteristics

All of the data in Figures 1 and 2 have been arranged so that the zero point on the abscissa represents the time of onset of the movement. The question naturally arises as to how the reaction time varies with the separation of the targets. For such information it is better to randomize the presentation of the targets to avoid the interaction of successive items. For this reason a second series of measurements on this subject and two series of measurments (of reaction time only) on a second subject were obtained. All these data showed the same general trends and so only the means of all four series are presented in Figure 4. In this figure the ordinates are reaction time values (in seconds) and the abscissas are values for the change in the stimulus to accommodation in diopters. The value of approximately 200 milliseconds obtained for reaction time is in good agreement with Allen's data obtained by photographing images of lights reflected

from the corneas[4-6] and is fairly typical of the data for a simple visual reaction time experiment. It is clear that there is no very obvious change in reaction time with increase in the target separation. The differences between reaction time in the far-to-near shift and near-to-far shift seem to show no constant trend.

Figure 4 shows, also, the data for the durations of the movement as a function of the target separations. These data emphasize the extremely long time that it takes for these movements to be completed. Large differences between the shifts from near to far and far to near appear at the smaller target separations—the duration of the far-to-near shift being much shorter than the near-to-far shifts. This is what Clark has also reported.[7] For the larger target separations, however, this difference seems to disappear. The surprising features in both sets of data are not in these trends which may be merely chance variability but in the amazingly small range of variation in the durations of both types of movements over the wide range of target separations used. This suggests that the movements, in spite of the extremely long duration, would be difficult to classify in Stetson's[8] terms as slow tension movements.

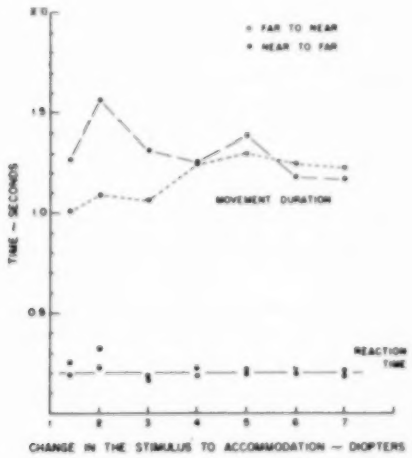

Fig. 4 (Alpern and Ellen). Time characteristics of the movement as a function of change in the stimulus to accommodation. The top set of curves show the variation in movement duration, while the bottom shows the variation in reaction time. The data for duration are the means of two sets of experiments at each stimulus level on the same observer as Figure 1 and Figure 2. The data for reaction time contain, in addition to these measurements, two sets of randomized presentations for a second observer.

Discussion

These results are in good agreement with previous experiments which have examined parts of this problem. The reaction time data and movement durations are in reasonable agreement with results obtained by Allen[4-6] and by Clark,[7] using photographic records both in the absolute values and in the relations of values obtained in the shift from far to near with respect to those obtained in the shift from near to far. Neither of these previous experimenters used a large variety of target separations and neither made an analysis of movements in terms such as those described above.

It is possible to examine Allen's data, however, and draw comparisons between these results and his. Before this is done, however, it is necessary to bear in mind that Allen's method of calibrating his record of eye positions is subject to serious criticism; for calibration data Allen rotated the arms of a haploscope through known magnitudes of arc and photographed the eye position using the subjective criterion of image unification as evidence that the eyes had moved an amount exactly equal to the stimulus. However, Clark[7,9] Peckham,[10] Tani, Ogle, Weaver, and Martens,[11] Westheimer,[12] and Alpern[13] have all found in such experiments that there can be large discrepancies between the record of the eye position and the convergence angle of the target.

Irrespective of the explanation for these discrepancies, and clear differences of opinion[11,13] persist on this point, it is apparent that such a method of calibration can certainly lead to an invalid estimate of eye position in the experiment of Johannes Mueller.

In addition, it should be pointed out that some of Allen's data of eye position have been averaged together with the zero point of the time axis as the time of onset of the *stimulus* rather than as the time of onset of the movement. The difficulties introduced in this process of averaging have been pointed out in the previous paper.[1]

With these possible sources of discrepancy between the two sets of data well in mind, however, the agreement between the results of the two experiments seems remarkably good.

In general, the shapes of the curves of eye movements obtained by Allen are quite similar to those reported here. Many of his curves (see particularly the data in[5,6]) show the maximum velocity in the early part of the movement with the velocity gradually becoming smaller as the eye approaches the maximum position after about one second from the time of onset of the movement, exactly as do those in Figures 1 and 2.

The data for some of Allen's subjects, however, show a gradually increasing velocity in the early part of the movement. Whether such data are to be attributed to one of the sources of error listed above, to differences in the stimulus condition or of the apparatus, or to individual differences in subjects can only be ascertained by further experimentation.

The values for velocity reported by Allen are higher than those obtained here but Allen's figures apparently refer to the maximum, rather than to the average, velocity. If one computes the values of the average velocity using the same criterion here employed, the order of magnitude of Allen's data is the same as those obtained in the present experiment. Some of his subjects do show higher values which may be different from the present findings by as much as a factor of four. It is possible that these discrepancies may be accounted for in terms of the artefacts in Allen's calibration arrangement since individual differences are likely to be especially apparent in the discrepancy between the stimulus to the movement and the photographic record of the position (Peckham). Differences in stimulus conditions are also important factors, and it is probable that differences in background luminance may be especially relevant. Quite likely individual differences in velocity of the movement in Mueller's experient also exist but it is not possible with the data at hand to make any positive statement as to the extent of this variability. Both sets of data are in reasonable accord with the values (25 to 10 degrees/second) reported by Dodge[14] for symmetrical convergence movements.

How can the time characteristic of the movements of the eyes in Mueller's experiment be explained? Currently there are two theoretic positions which supply an answer to this question.

The first theory stems from Dodge[14] and, rephrased in modern concepts of movement patterns as developed by Stetson,[8] suggests that this movement is a slow tension movement. The extremely long duration of the movement which is superimposed on a high level of background noise is especially suggestive of this theory.

A second hypothesis, recently suggested by Alpern and Wolter,[15] speculates that the final common paths for the movements in Mueller's experiment are the fine autonomic motor nerves which Wolter[16] and others have shown innervate the extraocular muscles (in addition to the typical somatic nerve fibers).

While a final decision between these speculations is not yet possible, evidence has been obtained that the notion of a slow tension movement is not very likely.

The present experiment demonstrated that the duration of the movement increased but slightly as the amplitude increased; but both the average velocity and the average acceleration increased considerably as the amplitude of the movement increased. These are not the characteristics of a slow tension movement whose velocities are relatively independent of the amplitudes of the movements. In addition it is well known that a slow tension

movement may be interrupted any time during the course of the movement and a new movement replace the old.

In a series of experiments in reaction time, Allen[6] found evidence that it is not possible to do this with Mueller's experiment. On two occasions his subject made an error (that is accommodated when he should have relaxed) but before the movement could be corrected the entire (wrong) movement had to be completed. The error movement in each case lasted over one second and the correct movement could only begin when the error movement was completed. This is more typical of the ballistic movement, although the velocity is much too slow. All of this suggests the reasonableness of the hypothesis that the eye movement, in Mueller's experiment is mediated through the autonomic nervous system and its much slower responding components. The value of the latter speculation in the explanation of the damping movements of the fixating eye in this experiment has already been emphasized.[1]

These experiments have pointed out some of the characteristics of the eye movements in the experiment of Johannes Mueller. Some of this information throws no obvious light upon the relation of accommodation to accommodative convergence per se. It is hoped, however, that when similar analysis can be made on movements obtained under a wide variety of different vergence stimulus conditions comparisons and contrasts will yield important information for theory construction.

Summary

A quantitative analysis has been made of the horizontal movements of the eyes of one subject in the experiment of Johannes Mueller, in which the eye shifts its gaze from far to near on the same line of sight while the other eye is occluded. All movement occurs only in the occluded eye. Analysis of the movement in terms of latency, duration, amplitude, velocity, and acceleration characteristics as a function of the separation of the far and near targets is presented. Theoretic implications of the results are briefly pointed out.

University Hospitals.

Acknowledgments

It is a pleasure to acknowledge the kind assistance of Dr. H. Richard Blackwell and Dr. Wilfred M. Kincaid of the Vision Research Laboratories.

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Discussion

Dr. Gerhard A. Brecher (Cleveland): The problem which the authors investigated is a time honored one about which much solid factual information is available. The question arises, therefore, whether a reinvestigation was warranted and whether, in doing so, new facts were discovered or a new concept was evolved. The authors have made a valuable contribution in both respects.

In applying the relatively simple technique of recording the changes of the ocular resting potentials (the term ocular rotational potentials should be avoided) caused by eye movements, the authors could establish that the peak velocities of the convergence movements associated with accommodation are consistently only one tenth or one twentieth as fast as those occurring in saccadic movements. This finding as such is not new. However, new is the statement that the time course of the convergence movement is always the same, irrespective of the extent of the movement, and that the movement once being initiated must always be completed before another vergence movement can be started. Furthermore a new contribution has been made as to the interrelation of the fast (saccadic) and slow (convergence) movements. In Johannes von Mueller's experiment the slow movement was observed in the occluded eye and the fixating eye remained quiet whereas with normal asymmetric binocular convergence both the fixating and converging eye started with a fast (conjugate) movement which was then succeeded by a slow (converging) movement.

Unfortunately the mechanical inertia of the recording equipment used by the authors does not permit a sufficiently detailed analysis of the fast component and its interrelation with the slow one. Also the relatively rapid beginning of the so called slow movement could not be analyzed. The exact determination of the time course of the fast and slow movement within the first 100 milliseconds would therefore elucidate the relation of the two types of movement. The authors should be encouraged to continue their work in this particular direction.

With the complexity of the interrelation of the fast and slow eye movements previously described in the literature it is important that the authors extend their investigations to more than one subject. It may well be that individual differences may account for the discrepancies between the authors' and previous findings in the literature.

For an interpretation of the difference between the fast and slow ocular movements the authors suggest the hypothesis that the fast movements are mediated through an innervation of the extraocular skeletal muscle fibers by medullated somatic nerve fibers whereas the slow movements are mediated by autonomic nerve fibers. The dual innervation of skeletal muscle fibers by peripheral nerves and autonomic nerves has been a subject of many investigations in the past (Boeke). Recently, Wolter has described nerve endings on the striated muscle fibers of the external eye muscles which have the morphologic characteristics of sympathetic and of parasympathetic nerve endings. However, up to the present, a solid body of evidence has been accumulated that such dual innervation does not exist in the skeletal muscles of mammals. The concept that autonomic nerve fibers would cause contractions of skeletal muscle fibers has never been demonstrated and is contrary to the accepted views on the role of the autonomic nervous system. I believe that the authors are leaving solid ground in advocating this hypothesis without evidence from conclusive electrophysiologic experiments. I want to emphasize this point because the danger exists that this attractive sounding hypothesis is accepted at face value in the ophthalmologic literature unless strong caution is expressed.

These considerations concerning an interpretation of the findings should not distract from the fact that the work reported here has been well executed.

Dr. Kenneth N. Ogle (Rochester, Minnesota): I just wanted to ask Dr. Alpern a question. It seems to me, if I understand his experiment correctly, that he was dealing primarily with accommodative convergence, though there might perhaps be also a proximal factor at the same time. I wonder whether he also conducted his experiments so that fusional convergence also came in. Might not the total reaction time, or the total time required to make the eye movements, be faster under the influence of fusional convergence than under just accommodative convergence alone?

Dr. Francis H. Adler (Philadelphia): In teaching Hering's law and in applying it clinically, I have been constrained for some time (because of other data) to limit the definition of Hering's law very decidedly to *voluntary versions*. If one does that, then Hering's law remains valid, and it is such a valuable law for us who are interested in clinical data that I hate to see it thrown out the window. All one must do is to make the statement of Hering's law as follows—in all voluntary versions of the eyes the innervation which is sent down goes equally to the muscles of both eyes concerned with the movement.

The experiments that were shown this morning

do not concern purely voluntary movements, but they represent reflex activities. Further than that, they are not versions but vergences. Hering was well aware of the fact that his law seemingly broke down in vergences when one changed the gaze from a distant object to an object which was nearer in the line of sight of one eye, where the movement apparently had to be limited to one eye.

He answered that seeming paradox, at least to his own satisfaction, by saying that it was a combination of versions and vergences which, on one side, were additive and on the other side one subtracted from the other, so that one eye alone carried out the movement.

Dr. Mathew Alpern (closing): I want to thank all of the discussants for their very kind remarks. If I might speak to Dr. Adler's remarks first, I think I am in complete agreement with everything he said. I am not trying to throw Hering's law out the window—far from it. I am impressed by the way in which it seems to hold with such precision, under conditions of binocular asymmetric convergence. We tried to emphasize in this study the amazing difference in this respect with regard to the movements of the eyes under binocular asymmetric convergence, and simple far and near shift in the gaze monocularly.

I point this out because I think this is a fruitful area of physiologic study as to what is going on here. There ought to be ways of probing these things, particularly electromyographically. Since one has to deal (in so far as vergence movements are concerned) with human eyes, we hope very shortly to get into doing these two experiments under conditions of human electromyography. This perhaps may throw additional light on the very pertinent problem that he has raised.

In regard to Dr. Ogle's question, we have some data in regard to conditions in which fusional factors as well as proximal and accommodative vergences are in play; and although I didn't bring the slide with me, what data we have seem to show remarkable similarity in the shape of the curves. That speaks only with reference to the raw data. I haven't made any systematic analysis of the corrected data in a way that might show up small differences, but in so far as just grossly looking at the raw data is concerned, one does not see any obvious differences in these types of movements. I hesitate to commit myself to that extent with the few data we have, but this at least is what we know at the moment about this matter.

With regard to Dr. Brecher's remarks, I must emphasize that the speculations we have made here are admittedly unconventional, and I would be the first to agree with him that they certainly ought not be taken for granted. Perhaps, their only advantage is that they do submit themselves rather easily to empirical testing.

We have made and are making empirical tests of this problem in our laboratory at the present time. Whether or not the speculations will withstand such tests, or whether we will have to come up with additional (or different) hypotheses, only time will tell.

SOME PHYSIOLOGIC CONSIDERATIONS OF HEREDITARY MACULAR DEGENERATION*

RODNEY D. STEINMETZ, M.D., KENNETH N. OGLE, PH.D., AND C. WILBUR RUCKER, M.D.
Rochester, Minnesota

INTRODUCTION

Hereditary degeneration of the macula lutea is characterized by the incidence of familial bilateral degenerative changes occurring in the macular area of the ocular fundus. At times the lesions are linked with cerebral involvement in the form of mental retardation, but other associated anomalies are uncommon. As a rule the inheritance is recessive, but there are a number of family records in which the macular lesion appears to have been inherited in a dominant manner. The modes of inheritance and the clinical course of the disease have been adequately described in the early literature by Behr[1] and by Sorsby,[2] and more recently by Sorsby and Davey[3] and Davis and Hollenhorst.[4]

Although most authors assume that the basic lesion is a retinal cone degeneration, remarkably few physiologic studies have been made to clarify the exact nature of the defect. Those reports which are available are often difficult to interpret. Therefore, in the study to be reported in this paper, special emphasis has been placed on tests of dark adaptation, critical flicker frequency, and color vision on 11 of 12 living "affected members" of a large family in which a hereditary macular degeneration is inherited as a dominant trait. The results of the tests on three individuals, each of whom belonged to a different generation, will be considered in detail in order to illustrate the probable chronologic development of the lesion.

The family was discovered when a 31-year-old housewife came to the Mayo Clinic because of a gradual, progressive loss of visual acuity which could not be corrected by glasses. Her history revealed that other kin had a similar visual disability. Since most of the family lived in southern Minnesota, it was possible to examine 62 members of the family and to investigate in detail the visual function of 11 of the 12 living affected individuals.

The pedigree of the family which is the subject of this study is shown in Figure 1. It consists of 158 members, 27 of whom had inherited bilaterally symmetrical degenerative lesions of the ocular maculas. The Roman numerals in the lefthand column of Figure 1 denote the generation; the Arabic numbers identify the individual members of each generation. This pedigree demonstrates that the lesion is transmitted as an autosomal dominant trait with complete penetrance.

CLINICAL CHARACTERISTICS

Case histories were obtained from all the living affected members of the family. These histories show that the clinical characteristics of the condition occur in a consistent and predictable chronologic course.

Owing to the insidious nature of the symptoms, it was difficult to determine the exact time of onset of the disease. However, most of the patients thought that their visual acuity began to decrease about the time they entered primary school between the ages of six and 12 years, when either they experienced difficulty in reading from the blackboard, or a school nurse recognized decreased visual acuity in a routine examination.

All of the patients, except the two young-

* From the Mayo Foundation and the Section of Biophysics and Biophysical Research and the Section of Ophthalmology, Mayo Clinic and Mayo Foundation. This study is an abridgment of a thesis submitted by Dr. Steinmetz to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Ophthalmology. The Mayo Foundation, Rochester, Minnesota, is part of the Graduate School of the University of Minnesota.

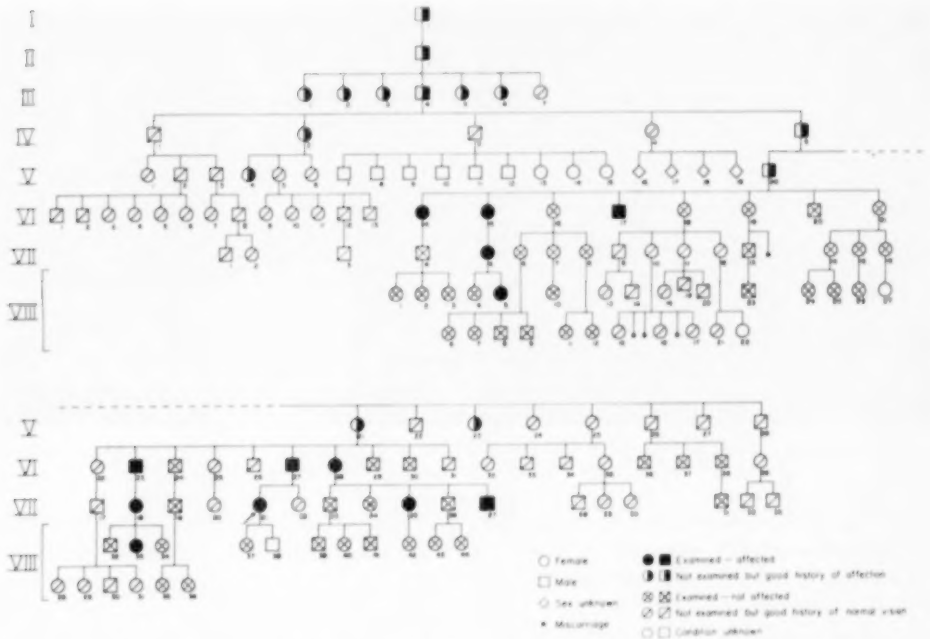

Fig. 1 (Steinmetz, Ogle, and Rucker). The pedigree of the family in which a hereditary macular degeneration is transmitted as an autosomal dominant trait.

est (VIII, 5 and VIII, 33), were uncomfortable in bright light to a degree consistent with the extent of the lesion. This symptom of photophobia was particularly annoying to the older patients, the majority of whom wore very darkly tinted sun glasses. The photophobia is similar to that experienced by the congenitally color blind, and this suggests clinically that the patients with the macular degeneration may have a generalized decreased function of the cones, as do those patients with achromatopsia. The patients in our study also claimed that their visual acuity was better in dim light than in bright light. The photophobia and day blindness were usually first experienced at about the same time, a few years after the decrease in visual acuity was first noted.

Defective color vision was also uniformly present in the affected individuals, but this was, however, the last visual symptom noticed by them. This result is contrary to the findings of Sorsby and Davey,[3] who reported that the defect of color vision was the earliest recognized feature of dominantly inherited macular degeneration.

Ophthalmoscopically, the macular lesions are first visible when the patients are about 35 years of age. The maculas of the 12-year-old girl (VIII, 33), as Figure 2 shows, appear normal. (The black dot in the center of the photograph is an artifact caused by a dust particle on one of the lenses of the camera.) The macular areas assume a slightly mottled appearance as shown in the fundus photographs (fig. 3) of the 39-year-old patient (VII, 18).

Each macular region of the 63-year-old patient (VI, 27) (fig. 4) exhibits a sharply delineated area about one disc diameter in size, in which there is apparently a destruction of the pigment and choriocapillaris layers. Sclerosed choroidal vessels are easily visible in the affected areas.

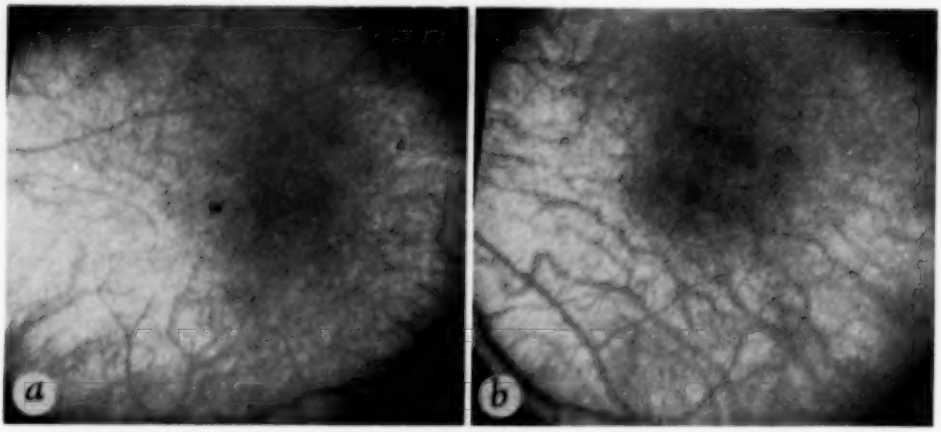

Fig. 2 (Steinmetz, Ogle, and Rucker). The macular area of each fundus of the 12-year-old girl (VIII, 33) appears normal. (*a*) Right eye. (*b*) Left eye.

In two collateral branches of the family in generation VI, it was noticed that for individuals about 60 years old the macular lesions appear different. The macular areas of two patients (VI, 27; VI, 28) in the one branch exhibit sharply delineated atrophic areas (fig. 4). Three patients (VI, 14; VI, 15; VI, 17) in the other branch, however, show a coarse pigmentary mottling in each macular region (fig. 5). Although the difference in the pattern of these areas is consistent, it should probably be considered a variation of the expression of the trait and not a true mutation. A similar situation was noted by Sorsby and Davey,[3] where one branch of a family showed more mottling in the macular areas, while the other branch exhibited an extensive and severe atrophic pattern. These authors hesitated to consider this variation a mutation of the macular dystrophy, but on the other hand they remarked that "it was equally unsatisfactory to regard it as an entirely independent entity."

In general there was a slow, relentless

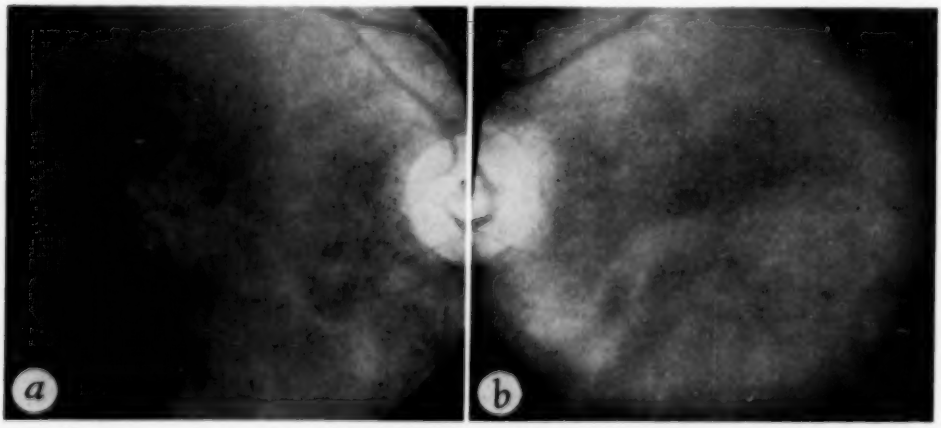

Fig. 3 (Steinmetz, Ogle, and Rucker). Slight mottling is shown in each macular area of the 39-year-old patient (VII, 18). This is representative of the earliest definite fundus change seen in this group with hereditary macular degeneration. (*a*) Right eye. (*b*) Left eye.

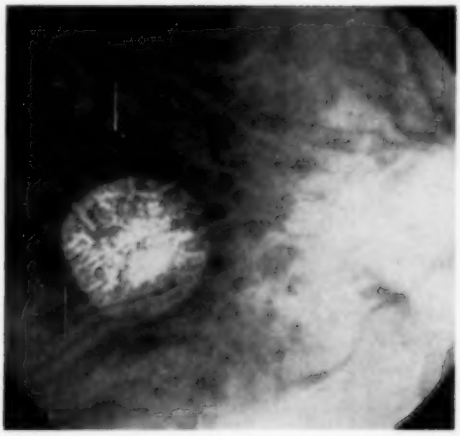

Fig. 4 (Steinmetz, Ogle, and Rucker). The sharply delineated atrophic macular lesion of the right eye of the 63-year-old patient (VI, 27) is characteristic of the branch of the family which contains members (VI, 27) and (VI, 28).

progression of the disease. However, two of the women in this family believed that their vision was substantially decreased immediately after the birth of their children. There were no associated mental or physical defects except that seven of the affected individuals had rather high refractive errors.

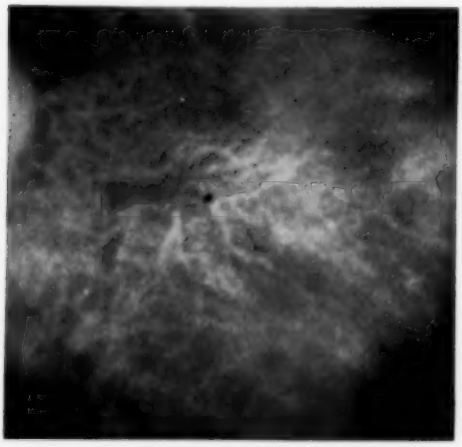

Fig. 5 (Steinmetz, Ogle, and Rucker). The pigmentary mottling shown in the macular area of the 69-year-old patient (VI, 14) is typically found in the branch of the family which contains members (VI, 14), (VI, 15), and (VI, 17).

The patients were all of average or above average intelligence.

It is interesting to note that all of the affected members had seen many specialists regarding their visual difficulties. Often they were accused of malingering or were diagnosed as having psychogenic problems because the visual disability was evident before the lesion at the macula could be seen with the ophthalmoscope. This diagnostic pitfall could have been avoided if an inquiry had been made regarding similarly affected kin.

Visual function tests

The results of studies of dark adaptation, critical flicker frequency, and color vision on three individuals (VI, 28; VII, 18; VIII, 33) (fig. 1), each a member of a different generation, will be considered in detail as typical of the probable chronologic development of the degeneration.

A. Dark adaptation

Published reports of studies of dark adaptation are infrequent in the literature on hereditary macular degeneration. Stargardt[5] reported that there was no change in the dark adaptation in a recessively inherited macular dystrophy. The finding that the light thresholds are increased, as described by Lutz,[6] was doubted by Stargardt[5] and by Sorsby and Davey.[3] Recently Sorsby and Davey[3] reported one patient with a dominantly inherited macular dystrophy who showed a restricted cone function. However, one affected sibling showed a normal cone function. Because in two other families the four adaptation studies made were normal, the authors concluded that in none of the three families which had dominantly inherited macular lesions was an obvious cone deficiency present.

1. *Instrumentation.* For our study a perimetric adaptometer (fig. 6) was used to determine the curves of light perception thresholds at different retinal angles. The test light consisted of a circular patch of white light projected by a suitable optical arrangement

Fig. 6 (Steinmetz, Ogle, and Rucker). The perimetric adaptometer used to determine light-perception thresholds in the course of dark adaptation.

on a screen on the arc of a perimeter 33 cm. from the patient's eye being tested. The intensity of this patch could be varied by a pair of circular optical wedges which could be turned equally in directions opposite to each other so that the transmitted light was uniform. The wedges were turned and controlled by a pair of Selsyn generators, one attached to the driving gear of the wedges, its companion mounted in a control box at some distance from the instrument. A circular scale on the Selsyn generator in this box provided a direct measure of the luminosity of the test patch. The angular size of the test patch used was a little less than two arc degrees. A suitable rotating sectored disc interrupted the light briefly about six times a second, a feature which aids the subject in remaining aware of the test patch. A fixation point was provided by projecting a red light through a one-mm. hole in the center of the perimeter arc. Subjects with defective macular fixation were instructed to look toward the center of an X-like figure made of four oblique self-luminous lines, drawn on cardboard and centered with respect to the red light.

2. *Procedure.* During the dark adaptation tests the left eye was occluded and the right eye was light adapted by having the patient look for three minutes at the screen of an X-ray viewing box 10 cm. away which had a white luminosity of 420 millilamberts as measured by the Macbeth illuminometer.* The direction of gaze was such that the portion of the retina being tested would be maximally stimulated. Since the hereditary macular degeneration is essentially identical bilaterally, only right eyes were tested.

Immediately after this light adaptation, the patient's head was placed in the instrument and the room was plunged into darkness. With the patient fixating the dim red light, the light threshold corresponding to a certain level of dark adaptation was determined every 30 seconds throughout a 10-minute test period. The luminance of the test patch was slowly increased from lower levels to that point at which the patch was first perceived by the patient. The determinations were discontinued after 10 minutes, since this was adequate to show whether or not a cone plateau in the threshold-time curve was present. Thus this procedure tested for the presence of cone function. The light thresholds during the course of dark adaptation for the first 10 minutes were determined at the fovea, and 7.5, 15, and 25 arc degrees nasal to the fovea in the horizontal meridian. The nasal field was chosen so that there would be no interference by the blindspot. A suitable rest period was provided between tests of different retinal positions.

Threshold studies made after 45 minutes in the dark tested the normalcy of the rods. The same instrument was used for determining these final thresholds of light discrimination at the fovea and then at certain positions on either side of the fovea in the horizontal meridian outward to the limits of the visual fields. The determinations were made at five arc degree intervals between the fovea and the 30 arc degree meridian, and then at 10 arc degree intervals to the limits of the visual field. At each position the illumination

*Macbeth Corporation, Newburgh, New York.

of the test spot was increased from nonstimulating levels to the point at which the light was first perceived.

3. *Results.* Typical results of our studies of dark adaptation are shown in the curves in Figures 7, 8, and 9, each representing one member of the last three generations. In the upper panels of the figures the logarithm of the threshold of light intensity just perceived is plotted against the time in minutes from the beginning of the process of dark adaptation. Also included on these graphs is the curve (interrupted line) representing the data of a normal 26-year-old subject and which is essentially the same as the curve for the mean of the data of 10 subjects with a normal dark-adaptation function. The lower left panel shows the log light threshold for different retinal positions after 45 minutes in the dark. The panel at the lower right pertains to the critical flicker frequency to be described in the next section.

In this study of nine of the affected patients, the cone segment of the dark-adaptation curve was missing in each of the seven patients older than 30 years. The two youngest affected members exhibited cone plateaus, but these appeared comparatively late and were of short duration. One patient (VI, 28) who had no lenticular opacities exhibited an elevated rod threshold after 45 minutes.

Since Hecht and Mandelbaum[7] showed that the range of normal curves was quite large, it was considered adequate, for the purposes of this study, to compare the form of the curve of normal cone function with that of the patients affected by the macular degeneration, rather than attempt to compare the thresholds quantitatively. The broken-line curves on the 45-minute-threshold charts represent the range of normal thresholds of 10 normal subjects obtained in a preliminary part of this study.

The 12-year-old patient (VIII, 33) (fig. 7) exhibited a delayed onset of light perception and also a very short cone plateau for all retinal locations tested. The light thresholds after adaptation in the dark for 45 minutes were within the normal range. This suggests some cone dysfunction with a normal rod function.

The 39-year-old mother (VII, 18) of this patient gave dark adaptation curves as illustrated in Figure 8. These show that the onset of light perception was delayed and, further, that the cone portion of the curves was missing entirely. However, the rod thresholds were within the normal range.

The 62-year-old patient (VI, 28) (fig. 9) exhibited a marked delay in perceiving the light at its highest intensity, and in the 10-minute dark adaptation curves no cone segment was present. The shift of the whole curve to the right was also noted for the foveal and for the peripheral areas of all the other patients of generation VI who were tested. All the affected members of the subsequent generations were less than 40 years of age, and none exhibited displacement of the curves. The displacement of dark adaptation curves to the right as a delayed response in older age groups had also been observed by Hecht and Mandelbaum[7] and by Birren and Shock;[8] they considered this to be a normal effect of age. The rod threshold of the patient (VI, 28) was elevated in the temporal portion of the visual field, thus suggesting that a decreased rod sensitivity was also present in this area.

The dark-adaptation curves of the 39-year-old patient (VII, 18) (fig. 8) are representative of those for patients with the mature lesion, and these closely resemble the curves determined by Sloan[9] on a cone-free eye of a patient with congenital achromatopsia. This curve indicates that the early lesion of the so-called macular degeneration of this family is not limited to the macula but is a gradual generalized cone degeneration of the entire retina. The lesion is most evident in the macular area because the cones are anatomically more abundant in that location. When the lesion is more advanced, as in the 62-year-old patient (VI, 28) (fig. 9), there may also be some associated decrease in rod sensitivity. The fact that a 63-year-old man

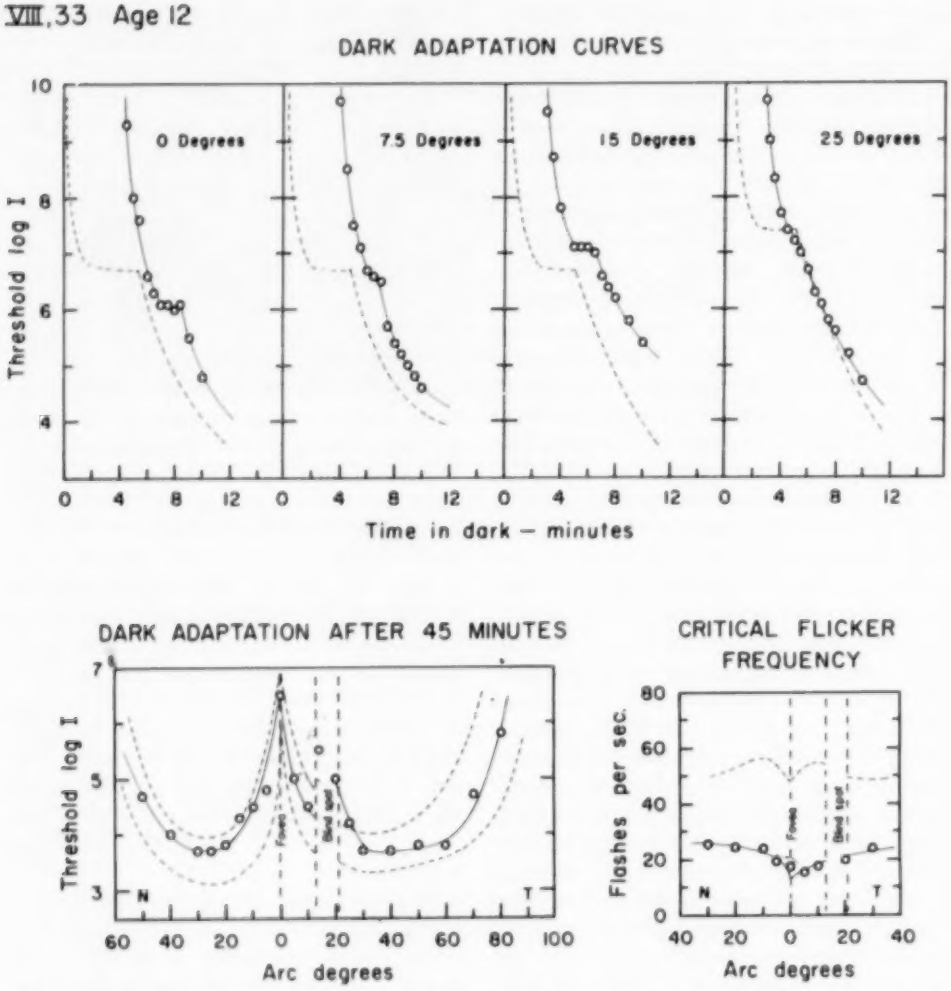

Fig. 7 (Steinmetz, Ogle, and Rucker). The results of determinations of dark adaptation and critical flicker frequency on a 12-year-old girl (VIII, 33) with hereditary macular degeneration.

(VI, 27) exhibited normal rod function indicates that there is not invariably a rod loss.

B. Critical flicker frequency

So far as we can find in a survey of the literature, no studies have been made of the critical flicker frequency in hereditary macular degeneration.

In tests made on totally color-blind patients, von Kries[10] found that the critical flicker frequency averaged 22 to 24 flashes per second as compared to the normal value of 60 flashes per second. Similarly, Hecht and associates[11] reported that the critical flicker frequency was lowered from the average normal of 55 flashes per second to a maximum of about 20 flashes per second under their testing conditions. Since totally color-blind persons supposedly have no cones in their retinas, the critical flicker frequency might be an important indication of the extent to which cone function is present.

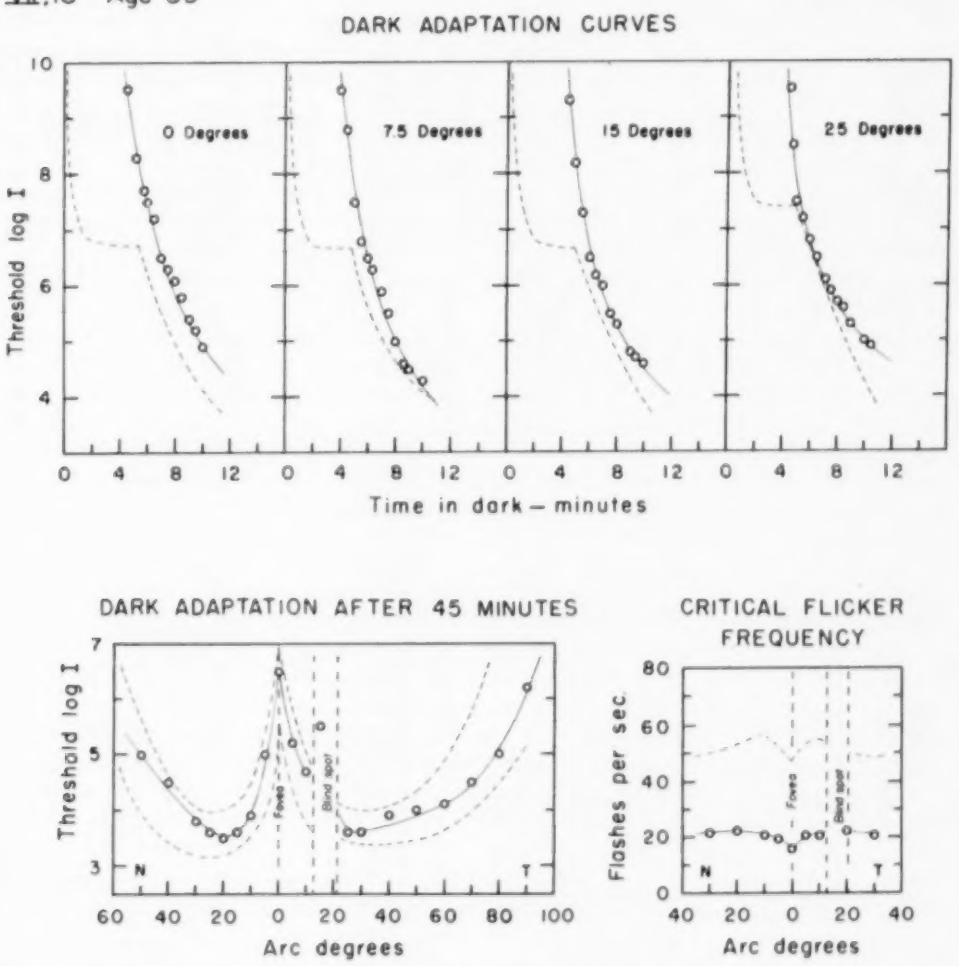

Fig. 8 (Steinmetz, Ogle, and Rucker). The results of determinations of dark adaptation and critical flicker frequency on a 39-year-old patient (VII, 18) with hereditary macular degeneration.

Certainly, at the scotopic levels of illumination generally used, as pointed out by Hecht and Verrijp,[12] cone activity is responsible for the critical flicker frequency.

1. *Instrumentation.* The apparatus for determining the critical flicker frequency consisted basically of the Strobotac,* an instrument used by others for such tests. This instrument gives a variable flash frequency with flash durations of 10 microseconds. It has a luminosity of about 15 millilamberts at the point of no flicker as measured by the Macbeth illuminometer. The instrument was set up at the eye level of the subject and at a distance of one meter.

A large sheet of white paper, in the center of which a hole 2.5 cm. in diameter (1.5 arc degrees) had been cut, was mounted in front of, and centered with, the Strobotac housing. On this sheet were painted single upper case

* General Radio Company, Cambridge, Massachusetts.

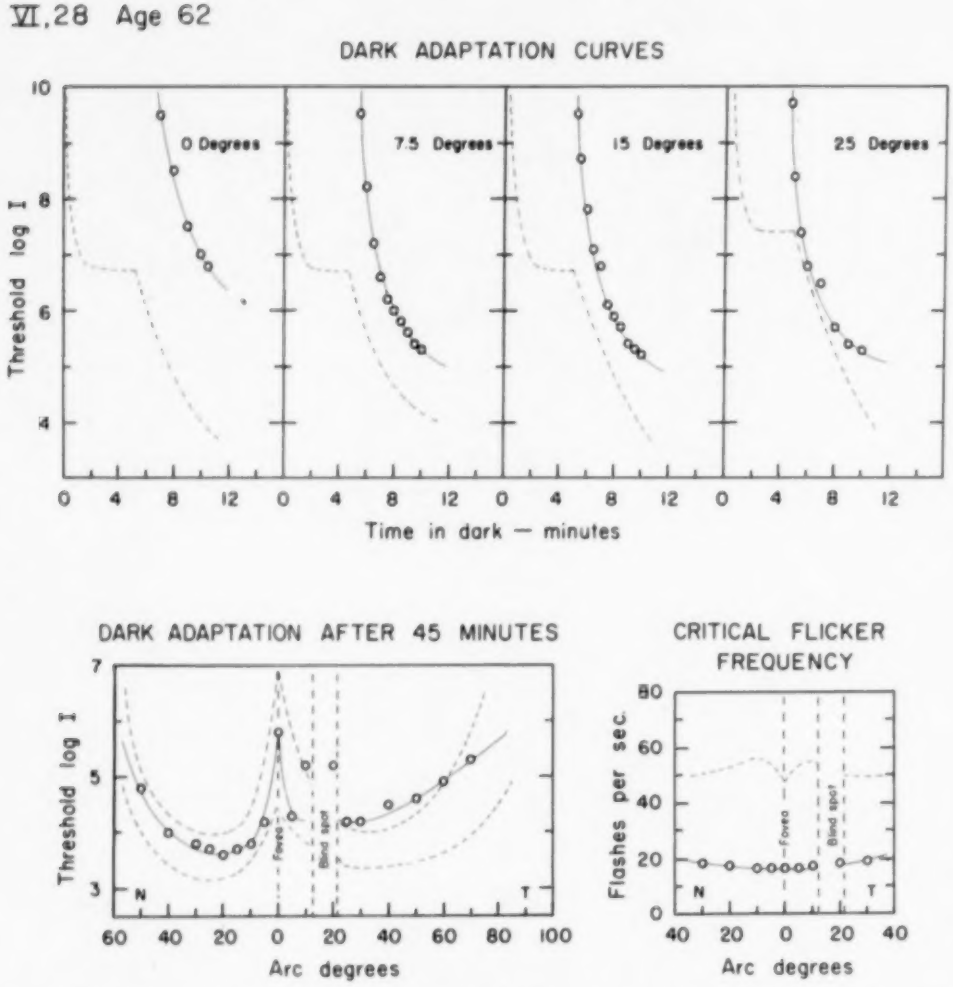

Fig. 9 (Steinmetz, Ogle, and Rucker). The results of determinations of dark adaptation and critical flicker frequency on a 62-year-old patient (VI, 28) with hereditary macular degeneration.

letters at intervals of 5, 10, 20, and 30 arc degrees on either side of the central target hole in the horizontal meridian, each letter subtending a visual angle of about 2.25 arc degrees. These letters were used successively as fixation points for determining the critical flicker frequency at several peripheral retinal angles. During the test the room was illuminated by four 40-watt lamps housed in an overhead light fixture which gave indirect illumination from the ceiling about 50 cm. above and behind the patient's head. Under these conditions the luminosity of the background sheet was about 1.1 millilamberts.

2. *Procedure.* The patients were placed before the apparatus and allowed five minutes to adapt to the background luminosity. Again, only the right eye was tested, since the inherited defect is known to be bilaterally

symmetrical. The left eye was occluded by a white translucent eye shield which allowed some light to reach the eye while totally obscuring recognition of detail. No refractive correction was used during our test in view of the work of Hylkema,[13] whose study indicated that, although the blurred retinal image had a lower intensity, it also covered a greater retinal area, thus making no difference in the critical flicker frequency with or without correction of refractive errors. Since the studies of Miles[14] and of McNemar[15] showed that it is not necessary to have an artificial pupil to standardize the size of the aperture, therefore none was used in this study.

Phillips[16] demonstrated that the retina quickly adapts to flicker, especially in the periphery, and recommended that the time of exposure be not less than one second or more than three seconds' duration. Therefore, in this test, exposures of two seconds were used and the subjects were required to state for each exposure whether the light appeared to be "flickering" or "steady." For each determination of the critical flicker frequency, the flickering light was first exposed with the frequency considerably below the fusion level. For each successive exposure, the frequency was slightly increased until the patient first reported that no flicker was seen. The macular area and then areas of the visual field at 5, 10, 20, and 30 arc degrees on either side of the fovea in the horizontal axis were tested in routine order. If the blindspot in the temporal field interfered, the patient was asked to fixate a point 10 arc degrees above, so that an area of the visual field just below the blindspot would be tested. It was not considered worth while to test beyond the 30 arc degree peripheral angle.

3. *Results.* The average normal values of critical flicker frequency of 11 normal subjects of comparable ages and the results obtained from 11 subjects affected with the hereditary macular degeneration are recorded in the table. For the same three subjects whose dark-adaptation curves were discussed, the results of the critical flicker frequency tests are shown also in the lower right-hand panels in Figures 7, 8, and 9. The critical flicker frequency for the affected group was markedly lower than that of the normals by an average of about 60 percent of the normal values at each peripheral angle tested, from the fovea to 30 arc degrees on the nasal and temporal sides. The generalized lowering of the critical flicker frequency by an amount comparable to that found in the congenitally color-blind persons suggests a widespread and uniform retinal cone degeneration in the members of this family affected by the lesion.

A closer examination of the table shows that one can average the critical flicker frequency for each individual to obtain a general figure for critical flicker frequency. This average is shown in the extreme right-hand column of the table. An inspection of these averages shows that there is a decrease in the critical flicker frequency for those individuals whose age is more than 60 years as compared to that for those less than 40 years of age. Statistically, this difference in the age groups studied is significant. It may also be significant that the decrease in the critical flicker frequency with age is greater for the larger nasal and temporal angles than for the smaller angles. For example, the difference between those patients over 60 years and those under 40 years is 6.6 for nasal angles 10 to 30 degrees, 3.4 for nasal angle five degrees to temporal angle five degrees, and 8.7 for temporal angles 10 to 30 degrees. Thus, some correlation is shown between the critical flicker frequency and the progression of the macular degeneration.

C. Color vision

Color-vision deficiency has been mentioned repeatedly throughout the literature on hereditary macular degeneration, but data regarding its presence have been so inconsistent that it is difficult to draw conclusions regarding its significance. Behr[1] and Sorsby[2]

TABLE 1

SUMMARY OF CRITICAL FLICKER FREQUENCY DATA (FLASHES PER SECOND)

A. Eleven normals

	Nasal Field					Temporal Field			
	30°	20°	10°	5°	0°	5°	10°	20°	30°
Average	52	55	60	57	50	56	58	53	52
Standard Deviation of Group	2.3	1.7	1.5	1.4	0.7	1.5	1.4	3.0	2.8

B. Subjects with hereditary macular degeneration

Kinship Number	Age (yr.)	Nasal Field					Temporal Field				Average
		30°	20°	10°	5°	0°	5°	10°	20°	30°	
VI, 14	69	19	20	20	18	17	19	20	18	18	18.8
VI, 15	68	17	17	17	17	17	17	15	15	15	16.3
VI, 17	61	23	27	18	18	20	17	15	13	12	18.1
VI, 27	63	18	20	21	20	18	18	18	19	18	18.9
VI, 28	62	19	18	18	18	18	18	18	19	20	18.4
VII, 5	34	23	22	20	18	17	21	22	23	23	21.0
VII, 18	39	23	23	22	21	17	22	22	23	22	21.7
VII, 21	31	26	28	21	18	18	20	21	23	23	22.0
VII, 25	31	38	38	32	27	28	28	32	38	39	33.3
VIII, 5	10	32	26	25	25	25	26	28	28	30	27.2
VIII, 33	12	27	26	17	20	18	16	18	21	25	20.9
Average		24	24	21	20	19	20	21	22	22	—
Percent decrease from normal		54	56	65	65	62	64	64	58	58	—

reported families in which some of the affected members had partial color blindness, whereas others had normal color vision. A family was reported by Halbertsma[17] which included six members with inherited macular degeneration with color blindness and six with defective color vision without signs of macular degeneration. In the present study all of the affected individuals had a disturbance of color vision, whereas the unaffected individuals had normal color vision.

All types of defective color vision have been reported for patients with this affection. "Defective red-green perception, daltonism, daltonism combined with decreased yellow-blue perception, and total color blindness" were all represented in the families reported by Behr.[1]

1. *Method.* In this study all the normal and affected members of the pedigree who were available for testing were examined, first, for color-vision deficiency by use of the Hardy-Rand-Rittler pseudo-isochromatic plates.* The Farnsworth-Munsell 100 hue test† was then used to check the results of

* American Optical Company, Buffalo, New York.

† Munsell Color Company, Baltimore, Maryland.

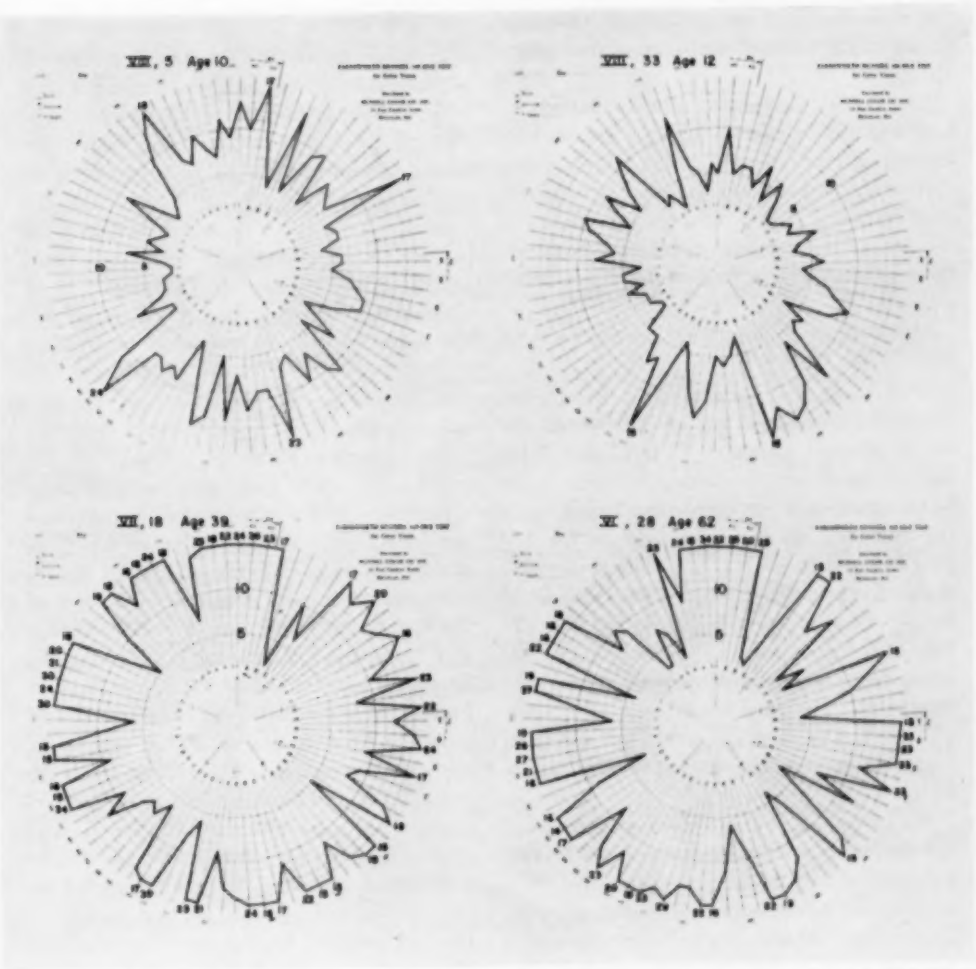

Fig. 10 (Steinmetz, Ogle, and Rucker). Graphic illustration of the results of the Farnsworth-Munsell 100 hue color tests on four patients affected by hereditary macular degeneration.

those patients who exhibited a defect of color vision on the plates. Both tests were administered under the illumination provided by the Macbeth New London easel lamp[3] according to the instructions of the respective designers.

2. *Results.* When examined with the Hardy-Rand-Rittler pseudo-isochromatic plates and the Farnsworth-Munsell 100 hue test, the older patients (VI, 28; VII, 18) (fig. 10) exhibited a very low discrimination for all colors. The Hardy-Rand-Rittler and the 100 hue tests (fig. 10) showed that the two youngest patients (VIII, 5) and (VIII, 33) were primarily tritanomalous, but that a milder red-green defect was also present. Sorsby and Davey's[8] report included two patients with macular degeneration who had similar defects of color vision. The three patients with hereditary macular degenera-

[3] Macbeth Corporation, Newburgh, New York.

tion described by Landolt[15] all had tritanopia as well as a lesser disturbance of red-green vision.

The early diminished discrimination of blue might be compared with the greater loss of perception of blue than red targets when testing the visual fields of patients with retinal lesions. This probably indicates that the lesion is a retinal degeneration rather than a defect originating in the visual pathways.

D. Visual fields

The visual fields as plotted on the tangent screen were obtained for all but two of the living affected members (VI, 17 and VII, 27). A small relative central scotoma could be demonstrated in each visual field with the exception of the right field of the 12-year-old patient (VIII, 33). The density of the scotoma varied from the one-mm. white target at two meters when testing the 12-year-old patient (VIII, 33) to the 18-mm. white target when examining the 69-year-old patient (VI, 14) and her 62-year-old cousin (VI, 28). The scotomas remained less than 15 arc degrees in diameter but became denser as the lesions progressed.

All of the affected members of generation VI showed contracted peripheral fields with the exception of the 63-year-old patient (VI, 27), whose fields were full to the one-mm. white target at one meter. Lenticular opacities can explain the contracted fields of two patients (VI, 14; VI, 15), but the media of the patient (VI, 28) were clear. Sloan[9] has demonstrated that, when eyes are adapted to brightness corresponding to that employed in clinical studies of visual fields, the thresholds of the light-adapted rods differ only slightly from those of the cones. Therefore, decreased function of both rods and cones would be necessary to give contracted fields. The contracted field of the 62-year-old patient (VI, 28) may be considered further evidence that a combination of cone and rod degeneration may occur late in the disease.

Summary and Conclusions

A family in which a macular degeneration is transmitted as an autosomal dominant trait with complete penetrance has been traced through eight generations. The pedigree includes 158 individuals, 27 of whom have inherited the defect.

Between the ages of six and 12 years, the patients first experience decreased visual acuity, which cannot be corrected with glasses. A few years later, photophobia and day blindness are experienced, and finally defective color vision is subjectively evident. When the patients are about 30 years old, it is possible to recognize the defect by ophthalmoscopy. First the foveal reflex disappears and the macular area has a granular appearance. There is a gradual change until the area is mottled with pigment when the patient is about 35 years old. When the patients reach 60 years of age in one branch of the family the macula has a sharply delineated atrophic appearance, but in the collateral branch a diffuse mottling persists. Functionally the two types behave similarly.

Physiologic studies on 11 of the 12 living affected members have been made by tests of dark adaptation, critical flicker frequency, and color vision, and by visual field studies. The dark-adaptation curves revealed a decreased cone sensitivity with normal rod function in younger patients and in one 63-year-old patient. A 62-year-old person with clear media exhibited a combined cone and rod degeneration. The critical flicker frequency was decreased an average of 60 percent of normal from the fovea out peripherally as far as 30 arc degrees in all age groups. Color vision tested by the Hardy-Rand-Rittler pseudo-isochromatic color plates and by the Farnsworth-Munsell 100 hue discs disclosed a general low color discrimination, with a tendency in the younger patients toward a relatively greater loss in the perception of blue. The older patients had a very low discrimination for all colors. Visual fields, as plotted on the tangent screen, re-

vealed that all subjects had relative central scotomas.

An analysis of the data according to age groups suggests that the lesion early in the course of its development is a cone degeneration. However, later the rods too may be affected.

Mayo Clinic.

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Discussion

Dr. Harold Falls (Ann Arbor, Michigan): Dr. Steinmetz has referred to different modes of inheritance of macular degeneration. At present there are three known patterns: simple autosomal dominant, simple autosomal recessive, and sex-linked recessive. Dr. Sorsby in his original writings on macular degeneration inferred, at least I so interpreted, that he believed a similar process was involved but varied in clinical expression depending on environmental and genetic milieux factors. He now, and I am certain that we do too, believes that there are many genes capable of altering macular physiology. There is great difference as to age of onset, course, prognosis, and clinical expression between families and types. Yet there exists, on the other hand, rather specific intrafamilial similarity. The manner in which individuals handle their daily stress and strain is important for I am convinced that certain hyperlabile types of individuals must have altered foveal physiology.

Investigators in the laboratory must be familiar with these variances of inheritance patterns and types of macular degenerations for no valid conclusions can be reached if the data are not pooled. Each individual is a law unto himself and we must in addition know something as to how his environment modifies his macular disease.

This also brings up the fact that it is necessary to study other members of the affected individual's family in order to be certain of the specific type of macular degenerative entity. Dr. Burian is cognizant of this and agrees that the application of pre-

cise visual physiology studies, such as this one, is necessary to accurate diagnosis. The simple expedient of examining the usual accompanying relatives is, for the clinician anyway, an extremely valuable adjunct in diagnosis since earlier and later stages of the entity may be seen.

For some time we have thought of the choriocapillaris, lamina vitrea, the pigment cell layer, and the rod and cone layer as a physiologic unit. Any damage to a particular layer, be it gene determined or environmental, eventually produces deleterious changes in the function and structure of the other divisions. Dr. Steinmetz and Dr. Ogle have in this family an excellent opportunity, by studying the younger as yet unaffected individuals, to determine the specific localization of the gene effect. The scalloplike edge of the boundary of the depigmented area suggests disease of the choriocapillaris in this family.

The matter of photophobia, in association with retinal pathology, has impressed me for some time. A rather large number of degenerative diseases of the eye, and specifically the retina, may be associated with the visible light and the ultraviolet spectrum. Mizuo's phenomenon, the retinal degeneration of the Galago monkey when exposed to light, the photophobia of the achromatopsia case, and so forth are examples. Leber was actually convinced that retinitis pigmentosa was a consequence of light toxicity in certain predisposed individuals. Xeroderma pigmentosa, epidermolysis bullosa also exhibit photophobia. The relationship of light to retinal disease is certainly worthy of the labor of the investigator and perhaps lies in the field of the enzyme chemist. Mammalian mutations which simulate human changes should certainly offer a wide and fruitful field of investigation.

Dr. Gerhard Brecher (Cleveland): It must be realized that in clinical investigations of the type described here the strict criteria of physiologic testing procedures are not always applicable. A reasonable compromise of what is mandatory for obtaining reliable data and of what the patient's patience can tolerate must be reached. In consideration of these limitations the authors have accumulated through their adequately designed testing procedures enough factual evidence to prove their points. The weakness of the color testing procedures employed (an anomaloscope should have been used) does not make the main body of the study less valuable. Since the work is well done and the conclusions are logical this investigation stands on its own merits.

Dr. Hermann M. Burian (Iowa City, Iowa): It is a particularly happy coincidence that we have had at this meeting two papers which complement each other so nicely. Both papers dealt with the physiologic investigation of patients in whom primarily one retinal mechanism was involved in the diseased process. Yesterday, Zeavin and Wald reported on their studies of patients in whom the rod function suffered first and foremost, whereas Steinmetz and Ogle reported on a condition in which the cones underwent degeneration.

I was particularly interested in the results of the investigation of dark adaptation study in these patients. In the normal individual one cannot obtain the rod threshold during the first eight or 10 minutes of dark adaptation, since it is far higher than the cone threshold. But if the cones are nonfunctioning one is entitled to attribute to the rods any thresholds obtained during this period. One is thus enabled in these abnormal eyes to study a function which is not accessible in normal eyes. Also, it would appear that the delayed response of these pathologic eyes in the scotopic phase is at least in part due to the fact that we are dealing here with rod function.

In connection with their dark-adaptation studies I should like to ask the authors two questions. One is technical: it is not clear to me where the patients fixated when the light was set at zero degrees. This is always a problem and I wonder how the authors solved it. The other question relates to the perimetric dark adaptation test. In the contours which they obtained after 45 minutes of dark adaptation the thresholds of the central retinal area fell within the range of normal thresholds, including the zero point. I do not understand this in the face of the fact that we are dealing with a cone degeneration. I wonder how the authors explain it, or whether I have misinterpreted their charts. In any event it would seem that it would be of great interest, if the opportunity presents itself, to test such patients also with lights of different wavelengths to differentiate further the rod and cone function.

A similar remark might also be made with regard to the critical flicker fusion frequency. With an arrangement permitting one to vary the intensity of the stimulating light it might be possible to investigate whether or not these patients present a cone curve and a rod curve for critical flicker fusion frequency—such as was shown to exist by Crozier and Wolff—and to analyze further the behavior of the different retinal mechanisms.

These additional possibilities are mentioned mainly to emphasize the importance of the field of study which the authors have opened up. The importance is twofold. These investigations not only shed light on retinal physiology and retinal pathology, but also are of clinical significance in that they help us in the understanding and diagnosis of some difficult cases in which a decrease in visual acuity cannot be accounted for by ophthalmoscopic findings, as was so well demonstrated by the beautiful fundus photographs of the younger patients which the authors showed us.

Dr. Rodney D. Steinmetz (closing): I would like to thank the commentators for their stimulating remarks.

The suggestions by Dr. Brecher and Dr. Burian regarding refinements in testing technique would be informative and should be employed in future

studies of this family. In the present investigation the testing techniques were simplified because it was necessary to get as much information as possible in a limited time. The members to be tested usually came in groups of three or four from farm areas some distance from Rochester and it was necessary for them to return home before sundown so their cows could be milked on time. Such are the problems that confront the country doctors.

Maintaining the patient's visual fixation during the tests was a major problem since all members of the family affected by the macular degeneration had relative scotomas. In addition to a red fixation light projected through a one millimeter hole in the center of the arc, a crosslike figure made by four oblique self-luminous lines which was centered with respect to the red light was used to help circumvent this problem. However, in spite of these efforts, the regulation of the patient's fixation was not perfect and, hence, the interpretation that rod thresholds in the macular area are normal should be made with reservation.

EYE MOVEMENTS IN AN ADULT WITH CEREBRAL HEMISPHERECTOMY*

DAVID VOLK, M.D., AND JAN H. BRUELL, PH.D.
Cleveland Heights, Ohio

Knowledge of cerebral areas or centers which have an influence on ocular motility comes, in part, from the observation of eye movements in patients with circumscribed brain lesions. We had an opportunity to perform extensive eye examinations on a patient whose entire right cerebral hemisphere had been removed surgically. The patient, a 39-year-old man, developed a malignant tumor in the right cerebral hemisphere. A right hemispherectomy was done. The incision extended through the corpus callosum. The globus pallidus, putamen, and caudate nucleus were removed, as well as a considerable portion of the thalamus. All that remained was the medial thalamus, hippocampus, and brain stem.

The patient made a remarkable recovery and survived five months, at which time regrowth of the tumor caused his death.

Postoperatively, he was found to have superior intelligence (verbal I.Q. of 125), and was completely lucid and co-operative. Five weeks prior to his death, numerous visual function tests were performed.

* From the Western Reserve University and Highland View Hospital, Cleveland. This study was supported by Grant G 1695 from the National Science Foundation.

CASE HISTORY†

The patient, a 39-year-old white man, was admitted to Western Reserve University Hospitals on October 14, 1954. He had been in good health until August, 1954, when he developed headaches which became progressively more severe.

On admission, positive findings were confined to severe papilledema, and some loss of fine movements of the left hand. Skull films, electroencephalogram, and ventriculogram gave evidence of a right frontotemporal mass. On October 18, 1954, a craniotomy was performed and a tumor was removed from the right frontotemporal area. Histopathologic diagnosis of the tumor was astrocytoma, grade four (glioblastoma multiforme). The patient had an uneventful recovery and was discharged on October 30, 1954, without demonstrable neurologic effects, except for a left superior quadrant homonymous visual field defect.

The patient was electively readmitted on November 25, 1954, for a right hemispherec-

† This patient was studied through the courtesy of the Neurological Service of Western Reserve University Medical School and the Department of Physical Medicine, Highland View Hospital, Cleveland, Ohio.

tomy and, on this admission, the only change in his examination since discharge was a recurrence of his papilledema. On November 26, 1954, a right hemispherectomy was done. This included the globus pallidus, putamen, and caudate nucleus, and a considerable portion of the thalamus. The major arterial supply was ligated just distal to its connection to the circle of Willis. Postoperatively the patient had a left hemiplegia, disturbance of body consciousness on the left with loss of localization of stimuli applied to the left side. After 60 days of convalescence, the patient became ambulatory with a left lower leg brace. He was able to localize stimuli to the general areas of the left side. Body image was described normally and he was able to differentiate touch, vibration, and pain stimuli with 60 percent accuracy, although he stated that all were variations of pain. On the 71st postoperative day, the patient was discharged.

The patient continued to improve as far as ambulation and self-sufficiency were concerned until three weeks before his last admission (April 10, 1955). At that time, he began to have pain in his right eye, with some loss of sensation over the first division of the right fifth nerve. Six days before admission, he began to have nausea and vomiting. On this admission, ventricular tap revealed increased intracranial pressure and increased protein (800 mg. percent). Right carotid arteriography revealed displacement of the left anterior cerebral and anterior communicating arteries, consistent with tumor regrowth. A ventriculogram revealed a space occupying mass in the right cranial vault. He was discharged without therapy and progressively deteriorated until his death on April 25, 1955.

Eye examination (performed on March 17, 1955)

The patient sat with his head tilted and turned slightly toward the left. The eyes appeared normal to inspection. There was a moderate degree of compound myopic astigmatism in both eyes which, when corrected with the patient's glasses, enabled acuity of 20/20 in each eye. The pupils were round, equal in size, and reacted normally to light stimulation and to accommodation. Tactile tension was normal. The media were clear. The fundi were normal except for some glial tissue on the discs, and on the blood vessels on and near the discs.

The rotations of the eyes in all directions, both to following and to command, were normal although the total extent of conjugate gaze to the left appeared to be very slightly less than to the right. The near point of convergence was approximately three to four inches. There was orthophoria for distance vision. Voluntary lid closure and spontaneous blinking were rapid and synchronous. Accommodation in the right eye was 3.5D. and in the left eye 5.0D.

Visual field studies revealed a left homonymous hemianopsia with the exception of the macular area in which vision extended two degrees to three degrees around the fixation point into the side of the hemianopsia. The vertical median was tilted approximately five degrees counterclockwise, the direction corresponding to the head tilt.

Optokinetic nystagmus tests were performed with a rotating drum. With the close surface of the drum moving to the left, the patient had normal optokinetic nystagmus; with the close surface moving to the right, the eyes moved partially to the right and remained there without nystagmus.

Dark-room experiments

The patient was seated in a totally darkened room and was asked to look all the way to the right. A flashlight, directed at the patient, was then switched on, and the eyes were seen to be in extreme right lateral gaze. The same test was repeated with the patient instructed to look all the way to the left, and the eyes were seen to be in extreme left lateral gaze.

In the totally darkened room, the patient was asked to look straight ahead. When the

flashlight was switched on, the patient's head was turned approximately 25 degrees toward the left, the eyes remaining parallel to the median plane of the head. An assistant then held the head in the straight-forward position, and the patient was instructed to look straight ahead. When the flashlight was switched on, the eyes alone were found to be turned approximately 25 degrees toward the left. The patient was again asked to look straight ahead in the totally darkened room, and the flashlight, which was placed approximately 25 degrees to the left, was flashed briefly. The patient reported the light to be straight ahead. If the duration of the light flash was increased, the patient corrected his erroneous judgment of the median plane.

In another test in the totally darkened room, a vertical luminous line was moved from left to right and from right to left in the frontoparallel plane of the patient, starting in each case 11 degrees from the objective median plane. The patient, whose head was held straight-forward by a head clamp, was instructed to report when the luminous line appeared straight ahead of him. The position which he indicated deviated 3.5 degrees (mean of 11 trials) to the left of the objective median plane, with an adjustment range from 8.5 degrees to the left to 2.3 degrees to the right.

When the patient adjusted a luminous rod to apparent verticality, his adjustment deviated 6.2 degrees counterclockwise from objective verticality (mean of six trials).

Discussion

The following is a discussion of only two of our findings, the first of which concerns voluntary eye movements.

When seen 111 days after hemispherectomy, the patient was able to move his eyes voluntarily in all directions as well as converge normally. These voluntary eye movements could be performed in a lighted room, as well as in a totally darkened room.

Voluntary conjugate movement of the eyes are thought to be induced by impulses arising in the frontal lobes of the brain, specifically the posterior end of the second frontal convolution, area 8 of Brodmann. Movements of gaze to the left have resulted from electrical stimulation in the vicinity of area 8 of the right cerebral cortex, and vice versa. Patients with acute lesions in this area in the right frontal lobe are unable to move their eyes voluntarily to the left, that is, to the side opposite the lesion, and vice versa. Such paralysis of gaze, however, does not persist. The fact that the paralysis is temporary suggests that other centers or areas are capable of compensating for the loss. The location of such centers or areas has not been established and one could postulate them to be in the same cerebral hemisphere, possibly in subcortical structures, or in the opposite hemisphere. The case presented here provides definitive evidence that voluntary eye movements in all directions, as well as convergence, are possible with only one cerebral hemisphere functioning. However, on the basis of the findings in this case alone, no conclusions can be drawn as to whether, in the normal individual, voluntary eye movements are controlled by both hemispheres simultaneously, or by one hemisphere at a time intermittently, or by one dominant hemisphere continually.

The second observation to be presented has to do with tonic innervation of the extraocular muscles. We use the term tonic innervation to encompass all innervation to the eye muscles other than voluntary.

The patient was seated in a completely darkened room and asked to look straight ahead. After the patient reported that he was looking straight ahead, a flashlight which was directed at the patient was turned on briefly and the position of the patient's head and eyes were observed. The first observation revealed the head turned approximately 25 degrees to the left, the eyes remaining parallel to the median plane of the head. If, in the darkened room, an assistant held the patient's head in the straight-forward position, and the patient was instructed to look

straight ahead, the eyes alone were turned approximately 25 degrees toward the left. On several occasions, the flashlight was held to the left in the 25-degree position and flashed briefly. When asked where the light was, the patient reported it to be straight ahead. Again in the completely darkened room, the patient was asked to indicate when a moving vertical luminous line appeared straight ahead of him. The position which he indicated deviated consistently to the left of the objective median plane.

In order to make understandable our interpretation of these observations, a few general considerations are now presented.

When one is told to look to the left, the extraocular muscles concerned with levorotation of the eyes receive a relative excess of voluntary innervation as compared to the dextrorotators. When one is asked to look to the right, the dextrorotators receive a relative excess of voluntary innervation as compared to the levorotators. When one is asked to look straight ahead in a totally darkened room, two possibilities present themselves:

1. Voluntary innervation to both the dextrorotators and levorotators is balanced, so that the eyes are held in position by voluntary effort, or

2. Voluntary innervation is at a minimum or absent, the eyes being held in position by tonic innervation.

It is our position* that the second alternative is the correct one, and that the experience of looking straight-ahead arises when there is no voluntary innervation. According to this view, when a subject is asked to look straight ahead the position his eyes assume in a totally darkened room reflects the tonic (involuntary) innervation to the extraocular muscles. An objectively straight-ahead position of the eyes reflects balanced tonic innervation to dextrorotators and levorotators. On the other hand, a levoversion of the eyes reflects a relative excess of tonic innervation of the levorotators, while a dextroversion of the eyes indicates a relative excess of tonic innervation of the dextrorotators. When our patient was asked to look straight ahead his eyes deviated consistently toward the left, indicating a relative excess of tonic innervation to the levorotators. If our interpretation of the factors responsible for the position of the eyes in a totally darkened room is correct, then our observations do not agree with the concepts presented in most textbooks.

According to present authoritative concepts, centers in the left cerebral hemisphere are responsible for tonic innervation to the dextrorotators of both eyes, and vice versa. Since our patient had only the left hemisphere, tonic impulses arising in this hemisphere should have caused his eyes to turn toward the right. The opposite occurred in our patient.

Our observations and the tests which we performed on this patient did not clarify the sources of the tonic innervation to the extraocular muscles, except that they could not have arisen in the right cerebral hemisphere. Similar tests upon patients who have undergone a hemispherectomy and who, prior to surgery, had normal eye function, will have to be performed to determine whether tonic innervation will cause the eyes to turn toward the side of the remaining hemisphere, or whether our findings were peculiar to our patient.

2460 Fairmount Boulevard (6).

* Experiments and observations supporting this view are presented in Bruell, J. H., and Albee, G. W.: Notes toward a motor theory of visual egocentric localization. Psychological Review, **62**: 391-400, 1955.

Discussion

Dr. Francis H. Adler (Philadelphia): A destructive lesion of the frontal oculogyric center on one side in man leads to a temporary paralysis of voluntary gaze to the opposite side. This is short-lived, however, and by the first or second week these movements are usually completely recovered. In

their discussion, the authors state that "following recovery from surgery the patient was able to move his eyes voluntarily in all directions." They do not state how soon after surgery this was found but the date of the examination given in the paper was March 17, four months after operation. It is to be expected that recovery would take place by this time.

This case provides evidence that the reflex movements of the eyes as well as the voluntary movements can be carried out with only one cerebral hemisphere functioning. To my knowledge this has never been demonstrated before. The influence of the frontal oculogyric centers on the occipital optomotor centers in area 19 has been studied, but the effects of total ablation of one hemisphere on the optomotor reflexes has never been shown in man and forms a valuable contribution itself.

It is also interesting to note that this patient had sparing of his maculas and retention of normal visual acuity, although one whole occipital lobe was removed. We usually explain sparing of the macula in occipital lobe lesions on the basis of a double blood supply to the cortical macula. This cannot be the explanation here.

I agree entirely with the author's statement (the second possibility) that in the dark the position of the eyes is maintained by the reflex mechanism until the patient wills to move his eyes, and that an object of attention moving from the straight-ahead position into the right visual field induces dextroversion by optomotor reflexes to the right turners. These impulses arise in the left occipital cortex, presumably in area 19. Similarly objects moving into the left visual field produce levoversion by optomotor impulses from the right area 19.

This patient should have shown an increase in tonus of his right turners, and his eyes should have been directed to the right under conditions of the experiment, but in reality he showed the opposite. I do not agree that this negates what I have said above and which is found in textbooks but merely points to the complexity of the experimental set-up we are dealing with. We should observe extreme conservatism in drawing conclusions from such data alone, and merely acknowledge that the experimental facts do not fit in with our present concept but do not disprove them.

Let me remind you that Hughlings Jackson, who was the great proponent of cerebral localization of function, pointed out that the responses which occur after removal of a part of the cortex cannot be interpreted as due simply to the absence of the ablated part, but also to abnormal responses from the parts which remain. That dictum also applies here. Further, there is the possibility that irritative lesions may have produced the picture seen here, since the eye examination was made five weeks prior to the patient's death, and three weeks before his death he had increased intracranial pressure, evidence of tumor regrowth by both arteriography and ventriculography, and other clinical signs.

Finally, recent evidence points to the fact that the cerebral centers for movements of the limbs do not actually initiate voluntary movements, and that the precentral gyrus is not the only ganglionic collection that is used for voluntary movements to the limbs. Penfield (Penfield and Wilder: Brain, 77: 1, 1954) has shown that the controlling impulses seem to flow upward to the precentral gyrus from some subcortical position, which he terms the "centrencephalic integrating system." These impulses reach the precentral gyrus, are modified and integrated there, and then proceed downward again to the bulbospinal gray matter on the way to the muscles. It seems quite possible to me that a similar complex organization may eventually be found for the oculogyric frontal centers.

If the authors of this very interesting and thought-provoking paper will agree that further evidence will have to be obtained before the phenomena they have observed can be satisfactorily explained we will be in entire agreement.

DR. JAY G. LINN, JR. (Pittsburgh): I have recently had under observation a patient on whom a hemispherectomy was performed one year ago. Dr. Volk has kindly mailed me a copy of his paper so I might perform identical tests on our patient and compare our findings with theirs.

The patient is a 16-year-old boy first seen at the Children's Hospital of Pittsburgh, when only 13 months of age. He had suffered from periodic convulsions for nine months and had a spastic paralysis of the left arm and leg. One year later an exploratory craniotomy revealed a brain cyst.

He was not seen again for 10 years at which time he was seen in the out-patient department because of convulsions which occurred three to four times a week.

In spite of some progress with walking and improvement of the hemiparesis his general condition progressively deteriorated. On July 1, 1955, at 15 years of age a craniotomy with right hemispherectomy was performed. The incision extended through the corpus callosum into the right lateral ventricle posterior to the splenium and then through the external capsule. The basal ganglia were not removed. The diagnosis was atrophy of the right hemisphere.

Examinations on numerous occasions since surgery have revealed a slight head turning to the right. A conjugate gaze paralysis to the right has remained. There is no follow movement beyond the midline but he is able voluntarily to rotate the eyes to the right with great effort (command movement) associated with gross nystagmoid jerks.

Visual field studies have revealed a left homonymous hemianopsia with macular splitting. The vision of the right eye is 20/20 and the left eye 20/60 with correction. Optokinetic nystagmus was present when the drum was rotated to the left but was absent when rotated to his right. It was impossible to demonstrate cogwheel movement satisfactorily.

The dark-room tests were performed as described by Dr. Volk and Dr. Bruell. When directed to look to the right his eyes were found to be straight ahead but when directed to look left his eyes were moved well to this side. When told to look straight ahead

the eyes were observed to be directed about 30 degrees to the left. When the light was flashed momentarily at a position 30 degrees to the left he accurately located the light on the left side.

When his head was held in a rigid straight-forward position, he always could determine when the vertical luminous rod was directly in front of him; he moved from his right side to the left. When the rod was moved from left to right, he located it as straight ahead when it actually was about 10 degrees to the left of the midline in about one of every three trials. In the other trials it was located accurately in front of him. He had little difficulty in placing the luminous rod in a vertical position.

Our patient differs in that the cerebral pathology destroyed the one hemisphere shortly after birth. He has therefore adapted to and compensated for his motor defects in a manner that is not possible in an adult. The basal ganglia were not removed.

The ophthalmologic findings in our patient differ in there being a conjugate gaze paralysis to the right. Dr. Volk and Dr. Bruell found a mild weakness of gaze to the left. Their finding is consistent with our knowledge of the normal motivation of conjugate movements from the cortex while ours is directly opposite. In spite of this discrepancy our dark-room tests were similar although the spatial orientation was a little better in our patient.

At first glance both cases seem to throw doubt on the accepted knowledge of the cortical functions in eye movements. Actually they do not contradict our previous knowledge.

It is well known that one cortex can take over many higher functions from the other. This is well demonstrated in the I.Q. testing of their patient. Obviously the same occurred in their case for voluntary ocular movements.

Our patient had a persistent and permanent conjugate gaze paralysis. This would indicate that the inflammatory and atrophic pathology of the right hemisphere at one time involved also the pontine center for conjugate lateral gaze. The very fact that the patient of Dr. Volk and Dr. Bruell retained good conjugate movements in all directions indicates the lower centers were not involved.

Dr. David Cogan (Boston): The observations on this patient with hemispherectomy are so pertinent to a series of patients which I once reported, that I am wondering whether the authors are aware of that series or whether they don't think it is pertinent to the observations.

The series I reported had the title of "Neurologic significance of lateral conjugate deviation of the eyes on forced closure of the lids."* Patients with unilateral cerebral lesions, upon forced closure of the eyes showed a deviation of the eyes to the side opposite the lesion, indicating under that condition a net tonic gain of contraversion.

That seemed to be a little surprising that the deviation was opposite the lesion. I thought it was an original observation at the time, but I found that some 30 years previously Bárány had also observed it in some epileptic patients. My tentative explanation for it was that, analogous to spastic hemiplegia, there was an increase in tone on the paralyzed side.

I have had the opportunity to examine three patients with hemispherectomies, and all three of them showed the marked deviation of the eyes to the opposite side. They also showed a consistent defect in the opticokinetic response.

When Dr. Adler was speaking it also occurred to me that while we do classically represent a contralateral representation of eye movements, it is true that the ocular motor centers are a little different from the other motor functions in the body, at least electrophysiologically, in that a homolateral deviation of the eyes from stimulation of the cerebrum does occur not infrequently.

Not many observations have been made on the frontal area, but Penfield, in his stimulation experiments on human beings, found homolateral deviation of the eyes from stimulation of the ocular motor areas in the occipital areas almost as frequently as he did contralateral.

So, I think one can make out a case for bilateral representation of the eye movements in the cerebrum more than one can for other motor functions.

Dr. David Volk (closing): I wish to thank the commentators for their criticisms, suggestions, and additions.

First, I would like to answer Dr. Adler's question about what we meant by the phrase "following recovery from surgery. . . ." All we meant to imply by the term "recovery" was that the patient appeared to have overcome the usual gross effects of major brain surgery. He was ambulatory and completely lucid when we examined him 111 days postoperatively. We agree with Dr. Adler that talking about recovery without specifying the time elapsed between surgery and the "recovered state" is not sufficient and, therefore, we have changed the text of our paper accordingly.

Dr. Adler also suggested the possibility that an irritative lesion might have been responsible for the increased tonus of the levorotators. Our observations did not lead us to believe that such was the case. We feel that an irritative lesion tending to produce levorotation would have increased the difficulty of performing and maintaining dextroversion, and possibly have resulted in slow nystagmoid movements on attempted right lateral gaze. Our patient had no difficulty in keeping his eyes straightforward in a lighted room, nor did he have any difficulty in performing and maintaining dextroversion.

I was not aware of Dr. Cogan's paper relating to our subject and I am sure it would have added to this presentation. In regard to other tests performed, they are included in the written history. Time limitations prevented a full presentation of the case at this meeting.

I wish to thank Dr. Linn for his efforts in repeating our tests on a case of hemispherectomy which he examined and for reporting his results at this meeting.

* Arch. Ophth. **39**:37-42, 1948.

The results of our experiments relating to tonic innervation to the extraocular muscles have led us to the interpretations presented in this paper. Our conclusions regarding eye position from tonic innervation do not agree with conclusions based upon observations on other cases with brain injuries. Further observations on cases of hemispherectomy should clarify the issues involved.

We feel that our theorizing regarding tonic innervation is of secondary importance and that the major contribution of this case lies in the observation that voluntary eye movements seem to be unaffected despite the absence of an entire cerebral hemisphere. Whether both hemispheres are of equal importance in this regard remains to be seen.

THE HYDRATION OF THE CORNEA*

II. The effect of the intraocular pressure

John E. Harris, M.D., Leta Gehrsitz, M.D., and Louise Gruber, B.S.
Portland, Oregon

It is now well established that an exchange of water[1] and sodium[2-5] occurs across the limiting corneal barriers namely the endothelial and epithelial surfaces. This exchange is most rapid across the endothelial surface. It is a reasonable assumption that the other major ionic constituents of the aqueous and certain organic constituents as well readily traverse the endothelial surface.

Because of this constant exchange of the corneal water and ionic constituents with those of the aqueous, a continuous elimination of fluid from the cornea has been postulated to explain the fact that the cornea normally is relatively dehydrated. This elimination would require an expenditure of energy and consequently be dependent on the metabolism of the cornea. Such a metabolic influence on corneal hydration has been demonstrated by a variety of techniques including the exclusion of oxygen[6, 7] and the use of enzyme poisons.[8] Perhaps the most conclusive demonstration of active elimination of fluid has been the observation that the corneal hydration and cation accumulation which occur when the eye is refrigerated at 0°C. can be reversed when the structure is subsequently incubated at higher temperatures.[9, 10]

The essential constituent excreted from the cornea is not known. The theory has been advanced that some cation such as sodium or an anion may be actively transported from the cornea[2] but there is no conclusive evidence which supports this contention or indicates the ion involved. However there is suggestive evidence that water is actively excreted from the cornea.[10]

In our studies of this problem we have employed the temperature reversal technique, one which has proved valuable in the study of factors controlling the cation balance and water content of the lens[11-13] as well as other cells.[14-16] The technique depends upon the fact that when the metabolic activity of the cornea is reduced by refrigerating the enucleated eye in a moist chamber, there results an influx of water, sodium, and potassium, and presumably other ions as well.[10] In respect to the ionic and water content then the cornea approaches equilibrium with its external environment (in this case the aqueous). When the metabolic activity is normalized by raising the temperature, water and cation move from the cornea and the corneal hydration and cation content approach the steady-state normally observed. During this latter phase a direct measure of water and cation elimination is possible and the influ-

* From the John E. Weeks Memorial Laboratory, Department of Ophthalmology, University of Oregon Medical School. Supported by Grant B-187 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda 14, Maryland.

ence of various factors thereon can be determined.

Since this experimental situation requires the use of the enucleated eye it becomes imperative to determine the effect of the intraocular pressure on the excretion of the water and cation which accumulates in the cornea during refrigeration. Previous observations had led us to believe that the intraocular pressure might be of some significance. First, at the end of six hours incubation at 37°C. when the eye was much softer than initially, the rate of excretion of water from the cornea was still rapid even though the normal hydration had been substantially achieved. Secondly, some experimental situations in which the intraocular pressure was raised by injecting fluid into the vitreous caused a decreased elimination of water and sodium from the cornea.

Experimental

Rabbit eyes were used throughout. Generally they were obtained from a slaughter house and transported to the laboratory in a moist chamber in the cold. The eyes were placed cornea up on a moistened piece of gauze in a tightly stoppered tube. When the intraocular pressure was artificially altered, the container consisted of a piece of glass tubing of one inch inside diameter, tightly stoppered at both ends with a rubber stopper. A hypodermic needle was introduced through the lower stopper, cannulating the eye through the optic nerve. Direct communication with a reservoir of a modified Tyrode's solution* was provided, the reservoir being maintained at a predetermined and indicated height. The air was replaced with a mixture of 95-percent oxygen and five-percent carbon dioxide and the eyes refrigerated at 0°C. After 40 hours one of the pair of corneas was analyzed immediately. The other eye was subsequently incubated at 37°C. for a period of six hours before analysis of the cornea.

* As previously described[11] but devoid of glucose.

For analysis the eye was placed on its corneal surface and hemisected. The vitreous, lens, and iris diaphragm were gently removed and the endothelial surface of the cornea lightly blotted to remove excess moisture. A central corneal section nine mm. in diameter was removed using a cork borer or surgical trephine. The corneal section so obtained was weighed immediately, dried to constant weight, dry ashed with the aid of a drop of sulfuric acid, and subsequently analyzed for sodium and potassium using a Baird Associates flame photometer with the internal standard.

The corneal water and cation *contents* are computed in terms of dry weight, water being expressed as moles per 100 gm. of dry cornea, and cation as mEq. per 100 gm. of dry cornea. The cation *concentration*, mEq. per 1,000 gm. of water, has also been computed in certain instances. Percent recovery is calculated according to the formula: percent recovery $= \frac{Cr - Ci}{Cr - Cn} \times 100$, where Cn is the content of the normal cornea and Cr and Ci are respectively the content of the cornea following refrigeration and following refrigeration plus incubation at 37°C. The percent recovery is thus an expression of that fraction of the water or cation which enters the cornea during refrigeration that is subsequently excreted from the cornea during incubation at 37°C.

Results

The water and cation contents and cation concentrations of fresh corneas and the changes in corneal composition which occurred in the enucleated but otherwise unmolested eyes when the temperature was reversibly altered are indicated in Figure 1.†

† The analyses of fresh cornea were done on specimens transported to the laboratory at room temperature, the sections being removed about one hour after enucleation of the eyes. Our previous experience[10] had indicated that such delay did not appreciably alter the corneal hydration. The values here listed, however, are somewhat higher than those of Davson.[9]

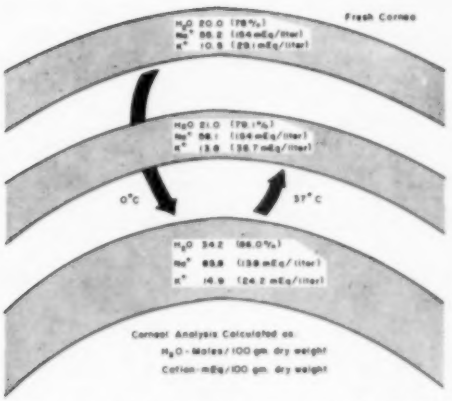

Fig. 1 (Harris, Gehrsitz, and Gruber). Changes in corneal composition of enucleated rabbit eyes after 40 hours refrigeration at 0°C. and six hours subsequent incubation at 37°C.

During 40 hours refrigeration the sodium content increased by some 55 percent and the water content by 71 percent. When subsequently incubated at 37°C. approximately 90 percent of the sodium and water that so entered the cornea were excreted.

The changes in corneal composition which occurred during refrigeration and during subsequent incubation at 37°C. of eyes with artificially controlled intraocular pressures are listed in Table 1.[‡] The excretion from the cornea of water in particular varied inversely with the intraocular pressures (fig. 2). The influence of the intraocular pressure on sodium elimination was less marked.

Of the three pressures studied the increase in sodium and water which occurred during refrigeration was least marked at the lowest pressure. However, it was substantially the same at 25 mm. and 40 mm. Thus, the pressure effect may not be simply due to a hydrostatic filtration of greater quantities of fluid into the cornea but may result from some effect on the secretory mechanism as well. More than likely a combination of these two factors accounts for the pressure effect.

During refrigeration the sodium (and potassium) concentration of the cornea of the unaltered enucleated eye fell and rose again to normal levels during incubation at 37°C. as the cornea became dehydrated (fig. 1). When the pressure was artificially maintained, however, the sodium concentration of the refrigerated eyes did not decrease but remained substantially that of fresh tissue and during subsequent incubation at 37°C. rose to above normal levels (table 1). In both situations this increase in corneal sodium concentration at 37°C. was approximately 15 mEq. per 1,000 gm. of water. The aqueous sodium normally lost to the lens and other structures during refrigeration was presumably replaced to a variable degree from the reservoir supply when the pressure was artificially maintained. This probably accounts for the higher sodium concentration in the corneas of these eyes. (The higher standard deviations probably attest to the variable diffusion of the fluid into the anterior chamber.) The current data are compatible with our previously enunciated thought that the cornea actively transports water.[10]

After six hours incubation at 37°C. the corneal hydration in the eye maintained at 7.5 mm. Hg was less than that of the unmolested enucleated eye, in spite of the fact that the unmolested eye was always softer than the eye at 7.5 mm. Hg. The difference presumably derives from the fact that a continuous supply of the reservoir solution is available to the cannulated eyes and may be due to a more stable pH or some other factor.

Discussion

The finding that the intraocular pressure alters the elimination of fluid from the cor-

‡ In the conduct of these experiments a leak in the system often occurred particularly at higher pressures. Under this circumstance water accumulated in the tube around the eye exposing the cut surfaces and epithelium to fluid. Our previous experience had indicated that the corneas of eyes so immersed gained water and cation excessively, presumably via the limbus. In experimental situations where this obtained, therefore, the eyes were discarded and not included in the results. For similar reasons, this technique was not adaptable to higher pressures.

TABLE 1

THE EFFECT OF TEMPERATURE REVERSAL ON CORNEAL COMPOSITION OF ENUCLEATED EYES WITH ARTIFICIALLY MAINTAINED INTRAOCULAR PRESSURE

Pressure	Procedure	No. of Eyes	Content			Concentration	
			Water	Sodium	Potassium	Sodium	Potassium
			Moles/100 gm. dry weight	mEq./100 gm. dry weight	mEq./100 gm. dry weight	mEq./1,000 gm. water	mEq./1,000 gm. water
7.5 mm. Hg	0°C. for 40 hr.	10	30.7±4.3 (84.5%)	84.0±12.6	13.2±1.9	152.1±10.2	24.5±5.4
	0°C. for 40 hr. plus 37°C. for 6 hr.	9	17.2±1.9 (76.2%)	56.0± 7.8	11.8±1.2	169.3±15.3	37.8±3.7
25 mm. Hg	0°C. for 40 hr.	12	34.3±5.0 (85.9%)	97.9±16.8	12.8±3.3	155.5±10.3	20.7±3.9
	0°C. for 40 hr. plus 37°C. for 6 hr.	15	19.5±2.7 (77.6%)	58.5± 5.5	12.3±1.2	172.2±12.7	35.6±6.5
40 mm. Hg	0°C. for 40 hr.	20	34.0±2.5 (85.9%)	95.9± 5.6	11.8±4.7	155.6± 9.7	20.6±3.3
	0°C. for 40 hr. plus 37°C. for 6 hr.	22	22.1±4.6 (80.0%)	64.9± 4.4	12.8±1.7	173.9±17.1	32.0±4.9

nea is not surprising. An increase in intraocular pressure in the normal eye is often accompanied by an increase in corneal hydration. The fact that the recovery is fairly substantial at 40 mm. Hg fits the clinical picture. Generally, in the undamaged eye little change in corneal hydration is seen at this pressure. The results also affirm that which has been suspected, that if the intraocular pressure can be sufficiently lowered, corneal hydration can be further decreased.

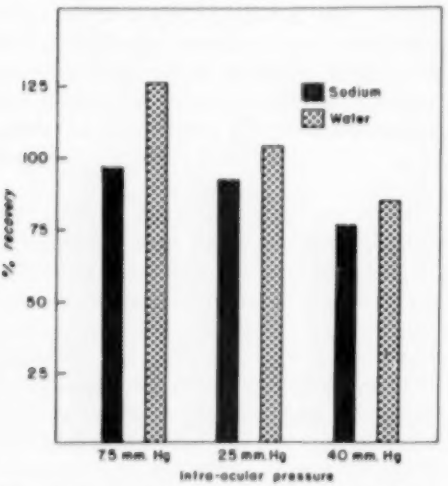

Fig. 2 (Harris, Gehrsitz, and Gruber). The effect of intraocular pressure on the elimination at 37°C. of water and sodium from corneas of previously refrigerated eyes.

It is well known that where the endothelial surface is damaged, however, the hydration even at lower pressures may become excessive. It would seem likely that this is due both to a failure of fluid excretion and to an increased rate of entrance of water and cation across the damaged surface. In this connection a comment on the action of acetazoleamide (Diamox) would seem appropriate. It has been reported that acetazoleamide is of value in reducing corneal hydration in individuals with endothelial dystrophy.[17] The exact mechanism by which this drug is of such benefit is not known. Unpublished experiments in our laboratory have indicated that acetazoleamide reduces the excretion of water and sodium from the cornea when injected into the anterior chamber. In this respect in the concentrations employed the agent is actually toxic to the cornea. The evidence would favor the view that any beneficial effect of this drug on corneal hydration results simply from a reduction in intraocular pressure.

SUMMARY

1. Water and cations enter the cornea from the aqueous humor when the isolated rabbit eye is refrigerated at 0°C. in a moist chamber.

2. This shift in water and cations (parti-

cularly sodium) is reversed when the eye is subsequently incubated at 37°C.

3. The excretion of water from the cornea of the previously refrigerated eye varies inversely with the intraocular pressure.

4. When the intraocular pressure is reduced from normal, the corneal water decreases.

3181 S. W. Sam Jackson Park Road (1).

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THE HYDRATION OF THE CORNEA*

III. The influence of the epithelium

John E. Harris, M.D., and Louise Gruber, B.S.
Portland, Oregon

Quite the equivalent of an axiom in ophthalmology has been the opinion that the maintenance of normal corneal hydration requires essentially only an intact and viable endothelium, the epithelium playing a subordinate and probably insignificant role. Indeed it has been the general clinical impression that removal of the epithelium is not followed by an substantial increase in the hydration of the cornea.

Maurice and Giardini,[1] however, found that the rabbit cornea swelled to approximately twice its normal value when the epithelium was removed in vivo. This swelling was substantially complete within six to 12 hours and, after maintaining a plateau for a period of one to two days, slowly decreased, approaching the normal value as the epithelium regenerated. Their figures suggested that a reduction in hydration did not occur until at least 50 percent of the epithelium had regenerated and that the swelling was still appreciable when a central area one third the diameter of the cornea was all that remained denuded.

In addition, Potts[2] found a rapid and considerable increase in corneal hydration when the epithelium was removed in vivo with 10-percent sodium desoxycholate and the eye bathed with a 1.35-percent sodium chloride. The results clearly indicate that an intact epithelium is essential for the maintenance of normal corneal hydration in vivo.

There are two obvious general possibilities by which the epithelium normally may prevent corneal swelling. First, it may simply provide a barrier to the ready entrance of fluid applied to its surface. Second, it may provide energy for the excretion of water or some ion either directly across the epithelial surface or across some other barrier. There is no question that the epithelium provides a certain barrier to the movement of water and physiologic ions and organic compounds. However, its possible role in the elimination of fluid from the cornea has been little explored. The excretion of water (or ions) from the cornea requires work, the energy for which must be provided by the metabolism of the cornea. The majority of the metabolic activity of the cornea rests in the epithelium.[3] Thus, an epithelial influence on the elimination of fluid from the cornea would be reasonably anticipated.

It is the purpose of the experiments herein described to determine the influence of the epithelium on the excretion of water and cation from the cornea. To this end the temperature reversal technique described in greater detail elsewhere has been employed.

Experimental

Rabbit eyes were used throughout. The experiments were conducted as previously described.[4, 5] The enucleated eyes were refrigerated in a moist chamber at 0°C. for 40 hours. One of the pair of corneas from the same animal was then analyzed for sodium, potassium, and water while the other eye was incubated at 37°C. for an additional six hours before similar analysis.

The epithelium was removed by various means:

1. Strong detergents such as 10-percent sodium desoxycholate and Zephiran (benzalkonium) chloride in two concentrations were employed.

* From the John E. Weeks Memorial Laboratory, Department of Ophthalmology, University of Oregon Medical School. Supported by Grant B-187 from The National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda 14, Maryland.

TABLE 1

THE EFFECT OF TEMPERATURE REVERSAL ON CORNEAL COMPOSITION OF EYES COMPLETELY DENUDED WITH DETERGENTS

Procedure	No. of Eyes	Content			Concentration	
		Water	Sodium	Potassium	Sodium	Potassium
		Moles/100 gm. dry weight	mEq./100 gm. dry weight	mEq./100 gm. dry weight	mEq./1,000 gm. water	mEq./1,000 gm. water
Fresh Cornea	93	20.0± 1.2 (78.2%)	55.2± 4.2	10.5±1.2	153.5± 5.5	29.1±3.2
Epithelium Intact						
0°C. for 40 hr.	30	34.2± 1.5 (86.0%)	85.8± 5.0	14.9±0.9	138.2±5.8	24.1±1.3
0°C. for 40 hr. plus 37°C. for 6 hr.	22	21.0± 1.8 (79.1%)	58.1± 5.4	13.8±1.1	154.3± 4.3	35.7±4.7
Epithelium Removed With 10% Sodium Desoxycholate						
0°C. for 40 hr.	8	60.4± 2.4 (91.6%)	145.0± 5.0	25.1±2.8	133.8± 2.4	23.5±1.7
0°C. for 40 hr. plus 37°C. for 6 hr.	8	57.8± 1.7 (91.2%)	152.1± 4.5	23.3±1.5	147.0± 3.9	22.8±2.1
1.0% Zephiran Chloride						
0°C. for 40 hr.	10	65.3± 3.4 (92.1%)	149.8±12.1	23.4±3.4	126.9± 6.8	19.8±3.2
0°C. for 40 hr. plus 37°C. for 6 hr.	10	54.7± 2.1 (90.8%)	145.9±17.7	22.0±1.4	144.7± 3.8	22.4±2.1
0.1% Zephiran Chloride						
0°C. for 40 hr.	10	34.1± 1.9 (86.0%)	86.4± 7.2	12.2±1.5	140.5± 7.1	20.0±3.2
0°C. for 40 hr. plus 37°C. for 6 hr.	10	36.1±12.5 (86.7%)	89.3±19.1	13.8±4.8	148.7±16.0	20.7±4.7

2. The epithelium was removed mechanically under a dissecting microscope, scraping with a sharp blade.

3. The epithelium was removed with the aid of a Thermophore at 140°F. (The heat loosened the epithelium but gentle scraping with a sharp blade was essential for complete removal.)

4. A caustic, two-percent tincture of iodine, was employed as a denuding agent.

Fluorescein was used on occasion to facilitate measurement of the area denuded. The dye did not appear to influence the results. Histologic examination revealed that complete removal was not obtained mechanically, a few peripheral cells generally remaining.

The endothelium was damaged by inserting a 27-gauge hypodermic needle through the limbus and stroking the central few millimeters of endothelial surface with the beveled edge. Only a fraction of the endothelium was purposely damaged. The area damaged, however, was not quantitated. As a control for these studies, the same needle was introduced at the limbus into the other of the pair of eyes, care being taken to avoid the endothelium. In either case when the needle was withdrawn a slight amount of aqueous invariably escaped from the anterior chamber.

Results are calculated in the manner previously described.[8]

RESULTS

When the epithelium was completely removed by gently rubbing the cornea with a cotton swab moistened with 10-percent sodium desoxycholate or one-percent Zephiran chloride the water and cation influx which resulted from refrigeration at 0°C. was profoundly increased as compared to the control eyes with intact epithelium (table 1). Excretion of sodium or water from these denuded corneas during subsequent incubation at 37°C. was nil (fig. 1). Apparently, the strong detergents caused considerable damage to the endothelium and permitted a more ready penetration of aqueous into the cornea. However when the epithelium was removed with the aid of 0.1-percent Zephiran* the elimination mechanism alone appeared to be altered (fig. 2). Cold-induced

* When Zephiran was applied in this concentration the epithelium was not readily loosened and gentle scraping with a sharp blade was also essential.

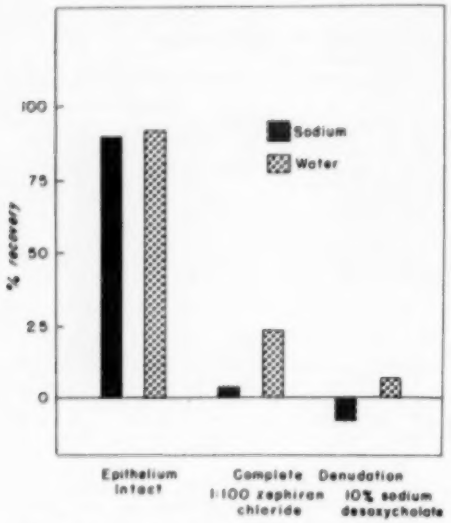

Fig. 1 (Harris and Gruber). The effect of strong detergents on the elimination at 37°C. of water and sodium from the corneas of previously refrigerated eyes.

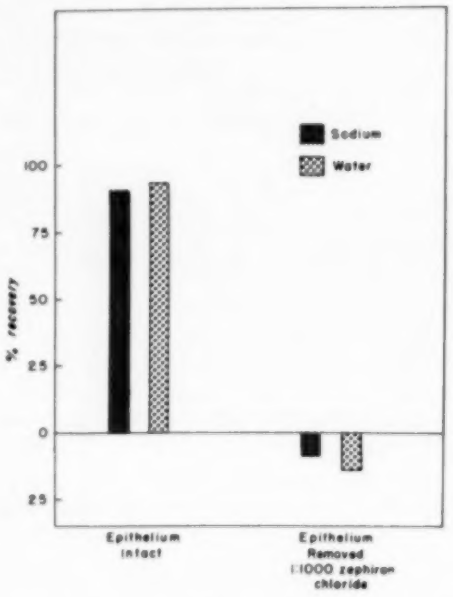

Fig. 2 (Harris and Gruber). The effect of 0.1-percent Zephiran chloride on the elimination at 37°C. of water and sodium from the corneas of previously refrigerated eyes.

corneal hydration and cation influx was substantially that of the unaltered eye. (In these experiments the results following incubation at 37°C. were quite variable for reasons which are not too clear; a likely possibility is the variation in the amount of Zephiran chloride which penetrated into the cornea.)

When the epithelium was totally removed either mechanically or with the aid of the Thermophore the cold-induced hydration and cation influx were not substantially different from that of the cornea with an intact epithelium (table 2). However a substantial but less than normal elimination of water and sodium from the denuded cornea during subsequent incubation was observed (fig. 3).*

When only the central epithelium was removed by mechanical means, no increase in cold-induced hydration over that normally observed was seen (table 3). In these instances excretion of water was unaltered (fig. 4). The results were the same when a central area four mm. in diameter and one seven mm. in diameter were denuded. (Assuming a corneal diameter of 10 mm. this would represent about 15 percent and 50 percent respectively of the epithelial surface.) Similar results were obtained when the Thermophore was used, removing a central area seven mm. in diameter (fig. 5). (The heat of the Thermophore did not appreciably alter the composition of the fresh cornea, the major difference being the expected reduction of potassium content when the epithelium was removed.) Lastly, removal of a central area seven mm. in diameter with two-percent tincture of iodine did not alter the degree of cold-induced water and cation influx or the subsequent excre-

* When the epithelium is completely removed, distillation to or from the cornea might be anticipated. In a vapor pressure equivalent to that of water, the water content of the cornea after six hours incubation at 37°C. without prior refrigeration was 22.2 ± 2.5 moles/100 gm. of dry cornea. When the vapor pressure was that of aqueous humor, the water content was 20.1 ± 2.2 moles/100 gm. of dry cornea, substantially that of fresh tissue. Significant distillation of water to or from the cornea under the experimental procedure, therefore, is unlikely.

TABLE 2

THE EFFECT OF TEMPERATURE REVERSAL ON CORNEAL COMPOSITION OF EYES DENUDED MECHANICALLY AND WITH THE THERMOPHORE

Procedure	No. of Eyes	Content			Concentration	
		Water	Sodium	Potassium	Sodium	Potassium
		Moles/100 gm. dry weight	mEq./100 gm. dry weight	mEq./100 gm. dry weight	mEq./1,000 gm. water	mEq./1,000 gm. water
Epithelium Removed Mechanically						
0°C. for 40 hr.	7	34.5±2.2 (86.1%)	90.1± 3.3	12.7±1.6	143.1± 6.3	20.1±2.2
0°C. for 40 hr. plus 37°C. for 6 hr.	8	24.6±6.0 (81.4%)	73.2±11.9	9.3±1.3	172.2±14.7	22.0±2.6
With Thermophore						
0°C. for 40 hr.	7	34.9±3.5 (86.2%)	90.9±11.5	13.9±4.3	141.4±11.3	21.6±7.0
0°C. for 40 hr. plus 37°C. for 6 hr.	8	24.3±2.8 (81.3%)	74.6± 9.9	10.3±2.8	169.1± 7.0	24.3±9.0

tion of water from the cornea. It is apparent that the usual clinical procedures used in denuding the cornea are not toxic to it.

When the peripheral two mm. of the epithelium were removed (with the aid of the Thermophore) leaving the central area intact, the excretion of water and elimination of sodium during incubation at 37°C. following refrigeration was significantly reduced (fig. 6). In this instance the cold-induced hydration was somewhat greater than occurred when the entire epithelium was removed or when the epithelium was intact (table 4). However it seems likely that this failure of recovery is largely a matter of failure of efficient excretion.

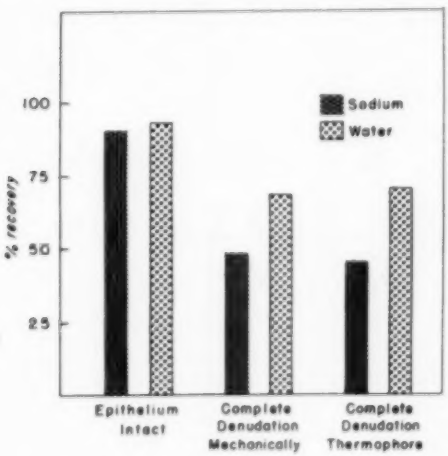

Fig. 3 (Harris and Gruber). The effect of complete denudation on the elimination at 37°C. of water and sodium from the corneas of previously refrigerated eyes.

For comparison data obtained when a central portion of the endothelium was damaged is included. The cold-induced hydration and cation influx was greatly increased when a segment of the endothelium was mechanically damaged (table 5). The fraction of water and sodium entering the cornea during refrigeration which was reversibly lost during incubation at 37°C. was correspondingly reduced (fig. 7). It is interesting, however, to note that the same quantity of water was excreted from the cornea with endothelial damage as from that of the control eye.

DISCUSSION

Changes in composition such as are reported here represent the balance between the entrance and exit of water and cation from the cornea (exchanging with a fluid of inconstant composition). As in all studies of this nature, it is difficult to be certain whether the particular experimental variable is influencing the permeability of the barrier or the secretory mechanism (if indeed the two can be separated). In the present studies it can be assumed as a first approximation that, where the water and cation content of the refrigerated cornea was substantially the same in the control as in the experimental eye, the degree of dehydration obtained during subsequent incubation at 37°C. provided

TABLE 3

THE EFFECT OF TEMPERATURE REVERSAL ON CORNEAL COMPOSITION OF EYES CENTRALLY DENUDED BY VARIOUS MEANS IN AREAS OF INDICATED DIAMETER

Procedure	No. of Eyes	Content			Concentration	
		Water	Sodium	Potassium	Sodium	Potassium
		Moles/100 gm. dry weight	mEq./100 gm. dry weight	mEq./100 gm. dry weight	mEq./1,000 gm. water	mEq./1,000 gm. water
Mechanically						
7 mm. area						
0°C. for 40 hr.	12	34.4 ± 2.0 (86.1%)	87.2 ± 6.4	13.6 ± 3.1	141.0 ± 7.5	21.6 ± 4.9
0°C. for 40 hr. plus 37°C. for 6 hr.	12	21.9 ± 3.2 (79.5%)	64.0 ± 8.5	11.7 ± 2.3	165.1 ± 9.9	28.5 ± 6.3
4 mm. area						
0°C. for 40 hr.	7	34.3 ± 1.8 (86.1%)	81.5 ± 10.8	12.4 ± 0.9	138.3 ± 6.6	20.1 ± 0.9
0°C. for 40 hr. plus 37°C. for 6 hr.	7	20.5 ± 1.3 (78.6%)	61.7 ± 6.1	11.6 ± 1.2	164.4 ± 6.9	31.0 ± 3.3
With Thermophore						
7 mm. area						
0°C. for 40 hr.	12	34.7 ± 1.2 (86.2%)	89.2 ± 4.1	13.5 ± 5.0	142.2 ± 6.5	21.7 ± 5.3
0°C. for 40 hr. plus 37°C. for 6 hr.	12	21.7 ± 4.1 (79.5%)	64.0 ± 8.5	11.5 ± 2.2	170.7 ± 10.6	29.4 ± 8.2
With Tincture of Iodine (2%)						
7 mm. area						
0°C. for 40 hr.	8	32.4 ± 2.6 (86.0%)	80.4 ± 8.8	15.4 ± 3.3	134.6 ± 7.2	25.4 ± 4.9
0°C. for 40 hr. plus 37°C. for 6 hr.	8	20.4 ± 1.6 (78.5%)	62.2 ± 5.9	11.1 ± 1.7	167.8 ± 10.5	30.0 ± 4.9

TABLE 4

THE EFFECT OF TEMPERATURE REVERSAL ON CORNEAL COMPOSITION OF EYES PERIPHERALLY DENUDED

Procedure	No. of Eyes	Content			Concentration	
		Water	Sodium	Potassium	Sodium	Potassium
		Moles/100 gm. dry weight	mEq./100 gm. dry weight	mEq./100 gm. dry weight	mEq./1,000 gm. water	mEq./1,000 gm. water
0°C. for 40 hr.	10	38.2 ± 0.7 (87.3%)	94.8 ± 7.2	14.8 ± 2.1	134.0 ± 5.9	21.3 ± 3.2
0°C. for 40 hr. plus 37°C. for 6 hr.	10	25.0 ± 2.0 (81.7%)	65.5 ± 6.9	13.1 ± 1.3	142.8 ± 9.3	29.5 ± 4.7

TABLE 5

THE EFFECT OF TEMPERATURE REVERSAL ON CORNEAL COMPOSITION OF EYES WITH PARTIAL ENDOTHELIAL DAMAGE

Procedure	No. of Eyes	Content			Concentration	
		Water	Sodium	Potassium	Sodium	Potassium
		Moles/100 gm. dry weight	mEq./100 gm. dry weight	mEq./100 gm. dry weight	mEq./1,000 gm. water	mEq./1,000 gm. water
Control (Limbus Punctured)						
0°C. for 40 hr.	8	35.7 ± 2.7 (86.4%)	80.8 ± 6.4	19.3 ± 4.2	127.3 ± 10.2	30.2 ± 5.3
0°C. for 40 hr. plus 37°C. for 6 hr.	8	19.1 ± 3.0 (77.5%)	55.9 ± 3.2	15.3 ± 2.5	164.8 ± 15.6	42.8 ± 1.2
Endothelial Damage						
0°C. for 40 hr.	12	43.3 ± 8.5 (88.5%)	95.8 ± 6.5	24.1 ± 3.0	123.0 ± 8.2	31.5 ± 4.8
0°C. for 40 hr. plus 37°C. for 6 hr.	12	28.0 ± 7.9 (83.4%)	73.5 ± 15.3	18.0 ± 3.3	151.3 ± 12.2	37.2 ± 7.0

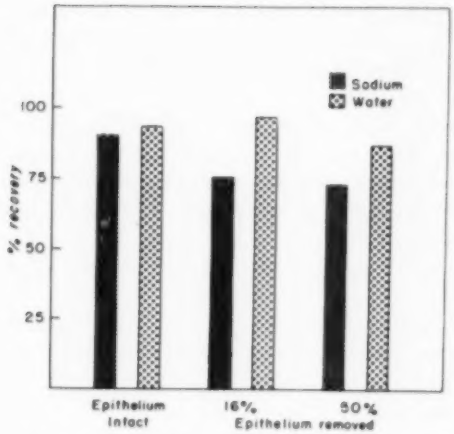

Fig. 4 (Harris and Gruber). The effect of central mechanical denudation on the elimination at 37°C. of water and sodium from the corneas of previously refrigerated eyes.

a measure of the effect of the experimental variable on secretory activity.

The strong detergents (10-percent sodium desoxycholate and 1.0-percent Zephiran chloride) are toxic to the cornea and probably not only increase the permeability of the endothelial barrier to the aqueous but in addition block the secretory mechanism. A lesser concentration of Zephiran (0.1 percent) appears to have only the latter effect.

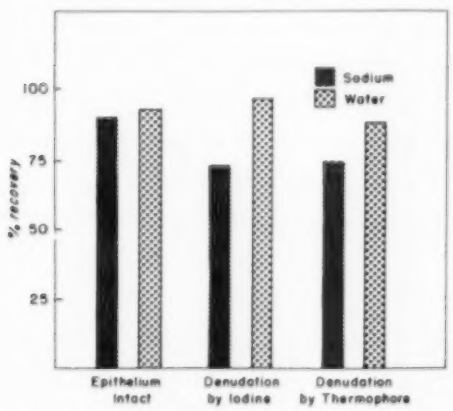

Fig. 5 (Harris and Gruber). The effect of central denudation by various methods on the elimination at 37°C. of water and sodium from the corneas of previously refrigerated eyes.

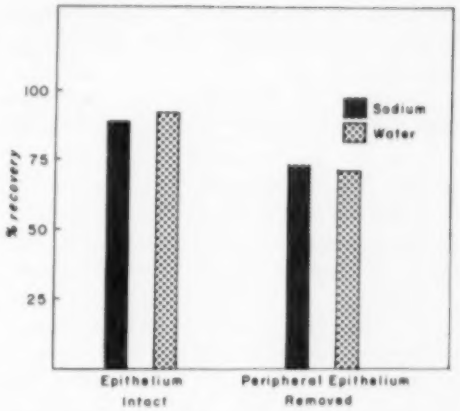

Fig. 6 (Harris and Gruber). The effect of peripheral denudation on the elimination at 37°C. of water and sodium from the corneas of previously refrigerated eyes.

Thus a separation of the permeability and secretory functions of the endothelium or some closely adjacent structure is feasible.

The present experiments have indicated that the epithelium plays a measurable role in the excretion of fluid from the cornea. The results of Davson[6] can be similarly interpreted. It is interesting that only the

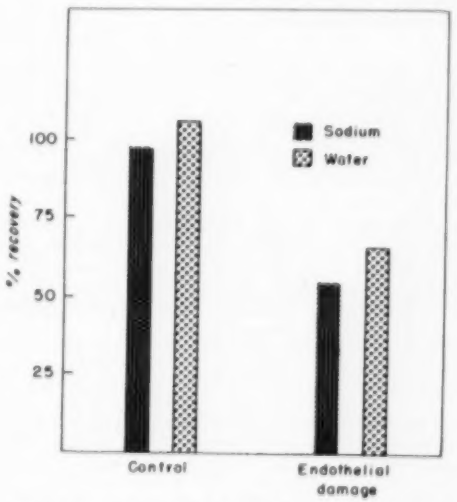

Fig. 7 (Harris and Gruber). The effect of partial endothelial damage on the elimination at 37°C. of water and sodium from the corneas of previously refrigerated eyes.

peripheral 50 percent of the epithelial area is active in this regard. The reason for this is not clear.

The data are compatible with the view that the endothelium plays the dominant role in the elimination of fluid from the cornea. Thus substantial secretory activity is preserved when the epithelium is completely removed. On the other hand a substance (0.1-percent Zephiran chloride) presumably toxic to the endothelium (or some closely allied barrier) can block the secretory mechanism completely.

In most of the experimental situations the elimination of sodium from the cornea during incubation at 37°C. lagged behind the excretion of water. The concentration of sodium in the cornea invariably rose. A similar increase in concentration of potassium was observed. The implications of these changes have been discussed and the suggestion made that such an alteration in composition is due to an active transport of water from the cornea.[4] The present results are compatible with this view without, however, negating the possibility of a secretion of sodium.

The present studies would indicate that the hydration which accompanies localized endothelial damage is due in certain measure to a break in the barrier and a more ready penetration of aqueous into the cornea. It is likely that in this instance the barrier break is a more important factor in corneal hydration than the defect in secretory activity.

Summary

1. The epithelium plays an active role in the excretion of fluid from the cornea. This activity is centered in the peripheral epithelium.

2. The role of the epithelium is fairly minor and the majority of the activity responsible for the excretion of fluid by the cornea rests in the endothelium or some closely adjacent structure.

3. Strong detergents are toxic to the cornea, increasing the permeability of the endothelial barrier to aqueous and blocking the secretory mechanism.

4. Denudation by heat or iodine are not toxic to the secretory mechanism.

3181 S. W. Sam Jackson Park Road (1).

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Discussion

Dr. Albert M. Potts (Cleveland): This is a very significant and satisfying piece of work, and needs little discussion. The results stand on their own merit.

The work done on the intraocular pressure was a necessary control, and the work done on the role of the epithelium was one that complements very completely the already published work in the literature. It shows, from an entirely different direction, that the corneal epithelium does represent a barrier. This was shown early by Dr. Cogan's group, and it was shown later by us, using radioactive sodium.

The problem of the endothelium, which was touched on, is a much more unique problem, because, if one observes denudation of the endothelium and its result, one sees that hydration oc-

curs only over the area denuded. This can be observed visually by looking at the clouding. This certainly is a picture which does not quite correspond to any physiologic mechanism that one can set up.

The emphasis that Dr. Harris puts on the possible excretion of water is one that we were very happy about, until we did experiments using tritium labeled water, which were reported at the Buffalo meeting of the Central Section last year, and which have not yet appeared in print. These experiments indicate that water, as measured by labeled tritium, penetrates very gaily through all these barriers in any direction, and that any water pump mechanism that one could postulate would have to work at a tremendous rate in comparison to a pump mechanism involving an ion.

The problem yet to be solved is this: If the ionic pump exists, what may this ion be? If the water pump exists, are the energetics in the cornea adequate to account for pumping of water with this very leaky system?

I tend to favor the former rather than the latter, simply because of the economics involved.

Dr. Bernard Wortman (Saint Louis): If one wishes to explain some of the phenomena of water and ion shift in the cornea on the basis of high metabolic activity, apparently the epithelium and stroma are capable of a great deal of enzymatic activity. We have applied microtechniques to the study of lactic, malic, and glucose-6-PO_4 dehydrogenases in epithelium and stroma. We found that epithelium is a very active, but labile, tissue. Stroma is also enzymatically active. Lactic and malic dehydrogenases are about 30 times more active in epithelium than in stroma. Glucose-6-PO_4 dehydrogenase was undetectable in stroma by our methods, although present in epithelium. The effect of lowering the temperature from 37° to 0°C. is such that all activities with the exception of lactic dehydrogenase in the epithelium drop to about five percent or less. Lactic dehydrogenase remained about 16 percent as active at 0° as at 37°C.

Dr. John E. Harris (closing): I certainly wish to thank the discussers for their remarks.

The localized swelling about a small area of endothelial damage is a very significant fact. For sake of simplicity a quite mechanistic view of corneal hydration has been adopted, one which envisages the cornea as a large cell with a superimposed "pump" of water or some ion. If such were the case however we should expect that a simple break in the endothelial surface would lead to uniform corneal swelling. This does not occur. I don't think we can dismiss this fact with the rather glib explanation that there is a resistance to diffusion of sodium and water through the corneal stroma. We are dealing with a much more complex mechanism than the thoughts that both Dr. Potts and I have expressed would indicate.

The fact that the epithelium is so metabolically active, of course, led us to undertake our present experiments. We could not quite believe that the epithelium acts simply as a physical barrier to hydration in vivo. The epithelium produces the majority of energy generated by the cornea, and when one asks oneself, to what end, he mainly thinks of regeneration of epithelial cells and maintenance of corneal transparency and hydration. Therefore the epithelium might be expected to have a considerable active influence on corneal hydration. We found that the epithelium contributed in some measure to the corneal dehydration mechanism but the effect was certainly not marked.

I might touch just briefly on the problem of whether water, a cation or an anion, is actively transported from the cornea. Our data suggest that it is water. The efficiency of a "pump" would conceivably vary, of course, with the rate of transfer or more specifically the rate of exchange of the substance that one is postulating to be excreted. As Dr. Potts has aptly stated, since the exchange of water is more rapid than that of ions, more energy would feasibly be required to excrete water than sodium or an anion.

However, that may not be strictly true, because the osmotic pressure of the cornea is probably very close to that of the aqueous. Thus, even though exchange of water across corneal surfaces is rapid, the energy requirement for active transport of water is not necessarily excessive.

RESIDUAL PROTEINS OF THE VITREOUS*

ANITA A. SURAN, M.S., AND W. K. MCEWEN, PH.D.
San Francisco, California

Most of the work demonstrating different components of the residual protein of the vitreous has been either microscopic or electron micrographic delineation of different types of fibers in the vitreous body.[1-5] Analytic differentiation of residual protein components has been done by several investigators. Balazs[6] showed that the residual protein is not distributed uniformly throughout the vitreous. Woodin and Boruchoff[7] and Gross, Matolsty, and Cohen[8] reported analytic data for various fractions of the residual protein. Dische and Zelmanes[9] analyzed the carbohydrate components of acid extracts of the residual proteins. Proteolytic enzyme analysis has been used by Bembridge, Crawford, and Pirie[4] and by Balazs and Varga.[10]

This paper details some experimental work on the analysis of the residual protein of the vitreous. There are two points of interest in addition to the partial analysis of the residual protein of the vitreous. One is the strong hygroscopicity of the residual protein, the other is an interesting mechanism of action of lytic enzymes on collagen.

METHODS AND MATERIAL

I. PREPARATION OF RESIDUAL PROTEIN

The vitreous were prepared by careful dissection and, after rinsing thoroughly and removing any extraneous tissues, the portion two to four mm. posterior to the lens was used. This excluded the zonular fibers. The residual protein of the vitreous was isolated by either of two methods, filtration or centrifugation. The filtration method consisted of (1) washing the vitreous for about a three-week period in frequent changes of distilled water with toluene and carbon tetrachloride as protection against bacterial contamination, (2) filtration of this washed material through loose nylon stocking (60 mesh, 15 denier), and (3) bringing to constant weight. This constancy is with respect both to drying and to hyaluronidase. The centrifugation method is by high speed ($\sim$100,000 $\times$ G) centrifugation to spin down the residual protein and is followed by several washings and finally drying to constant weight. The same weight of residual protein is obtained with or without hyaluronidase treatment.

Both methods yield an average of 10.5 mg. percent for the residual protein. This material, like many proteins, is hygroscopic. Because it is finally divided, its rate of water absorption is rapid. It will rapidly increase to 113 percent of its weight in 20 minutes when exposed to laboratory air and will reach equilibrium at about 120 percent of its dry weight. It will be noted that kangaroo tail tendon (a typical collagen) is also hygroscopic and will slowly increase in weight to 129 percent of its dry weight when exposed to air over a 16-hour period.

II. ENZYMATIC HYDROLYSIS

Wyeth's hyaluronidase† was used in a concentration of 10,000 TRU of hyaluronidase per 100 ml. of a 0.1 M acetate buffer (pH 5.4) in 0.15 M NaCl. One ml. of this buffer was used with each mg. of material to be hydrolyzed.

Tryptar, a brand of trypsin, was used as a 100 mg. percent solution in 0.2 M borate buffer (pH 7.8). One mg. of enzyme was used to hydrolyze from two to three mg. of material.

Collagenase‡ from Clostridium welchii was

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† Courtesy of Joseph Seifter, Wyeth, Incorporated.

‡ Courtesy of Wellcome Research Laboratories, Kent, England.

used as a 100 mg. percent solution in the same buffer as was used for the trypsin. One mg. of enzyme was used with one mg. of substrate.

Most hydrolyses were carried out at 37°C. for 16 hours.

III. Analyses

Hydroxyproline was determined by a slight modification of the method of Martin and Axelrod,[11] hexosamine by the method of Boas,[12] and nitrogen following the method of Grunbaum, Shaffer, and Kirk.[13] Both circular and two dimensional chromatograms were used. The solvent for circular chromatography and for the first dimension was n-propanol, H_2O, NH_4OH in proportion of 67:32:1. The second dimension was chromatographed with a solvent developed during the course of this work and is a mixture of benzene, ethyl alcohol (95 percent), and one-percent acetic acid in the proportions 20:85:30.

This solvent seemed more applicable than the usual phenol, collidine, leutidine types of solvents under conditions where no special ventilation was available. Good resolutions were obtained except in the case of methionine and valine which give one spot and a procedure is available for separating these two amino acids. The material on the chromatogram was visualized by the usual sprays: (a) Ninhydrin for amino acids (followed by Cu $(NO_3)_2$ for preservation, (b) Aniline phthalate for reducing sugars, and (c) Isatin chiefly for proline and hydroxyproline.

Results and Discussion

It is generally believed that trypsin will attack many denatured proteins. It has only weak action toward undenatured proteins.[14] The slow attrition of collagen by trypsin is shown in Table 1. It may be seen that when kangaroo tail tendon is subjected to repeated attacks of trypsin, with drying to constant weight between attacks, there is a slow, gradual loss of substrate.

TABLE 1

Percent remaining after trypsin degradations (composite data)

Number of Trypsin Attacks	Dried Intermittently		Kept Wet
	Collagen (Kangaroo tail tendon)	Residual Protein	Residual Protein
1	93	65	58
2	91	51	64
3	89	44	
4		40	64

When residual protein is subjected similarly to trypsin attack there is an early, large weight loss, quite different from the reaction of collagen.

After this initial drop in weight there is a continual weight loss that is similar to, but larger in amount than, when kangaroo tendon collagen is used as a substrate. It will also be noted that when the residual protein is kept wet there is little of this continual loss. The interpretation of these results is that there is a component of the residual protein which is more susceptible to trypsin attack than is the remainder of the residual protein. The remainder has slightly greater sensitivity to continuing trypsin attack than kangaroo tail tendon, probably by reason of its greater area. This difference in susceptibility to trypsin may be a rate phenomenon rather than a qualitative difference between the two substrates. Elimination of intermittent drying to constant weight appears to eliminate most of the gradual loss in weight due to trypsin activity with both substrates, which suggests that desiccation causes partial denaturation.

The material which is easily hydrolyzed by trypsin is called the trypsin attackable fraction and the remainder from this initial treatment is called the trypsin resistant fraction. The chemical analyses of these unpurified fractions are useful in showing trends and general characteristics but are not to be interpreted precisely. Table 2 gives the analyses of the whole residual protein and the trypsin resistant material. Our residual

TABLE 2

ANALYSIS OF FRACTIONS OF RESIDUAL PROTEIN

	Amount (mg. %)	% N	% Hydroxy-proline	Hexos-amine*	Sugars†
Residual Protein Preparation	10.5	11.5	11.3	4.0	+++
Trypsin Resistant Material	6.4	14.7	12.5	0.3	+++

* Analysis courtesy Mr. Henry Dix, Department of Pathology.
† Qualitative data from chromatography.

protein results are close to those of Woodin.[7] We show a slightly higher hydroxyproline content. The analysis of the trypsin resistant fraction is similar both to Pirie's[15] precipitated protein and to Matolsty's Vitrosin.[8] It appears that the hexosamine has been largely hydrolyzed but that other carbohydrate remains with this fraction. Although this resistant fraction appears analytically to be more collagenlike than the initial residual protein, it still differs considerably from the typical collagen analysis.

From these data it is apparent that the material hydrolyzed by trypsin contains the hexosamine, considerable hydroxyproline, and accounts for some 40 percent of the material.

The trypsin resistant fraction of the residual protein may be further degraded by a combination of repeated drying, trypsin, and hyaluronidase treatments or by collagenase. The hydrolysis is not complete as there is a small residue of 1.0 to 2.5 mg. percent. It is of interest to note that the hydrolysis of both collagen and the trypsin resistant fraction by drying and trypsin may be increased by treatment with hyaluronidase. This is shown in Table 3. The increased breakdown of both proteins after hyaluronidase treatments is another example of the importance of sugar in the collagen molecule as previously shown by Banga[16] and Jackson.[17]

The residue from collagenase treatments was hydrolyzed with acid and chromatographed. There was no proline or hydroxyproline although the other amino acids were present in this fraction.

TABLE 3

EFFECT OF HYALURONIDASE ON SUCCESSIVE TRYPSIN DEGRADATIONS

Treatment	% Remaining			
	Residual Protein	Collagen (Kangaroo tail tendon)		
	Dried Inter-mittently	Dried Inter-mittently	Kept Wet	
Trypsin 1	94	93		
Trypsin 2	90	91		
Trypsin 3		89		
Hyaluronidase 1	60	81		
Trypsin 4	36	77		
Trypsin 5		72		
Trypsin 6		62	85	

SUMMARY

1. The residual protein of bovine vitreous may be differentiated enzymatically into three components:

a. A trypsin hydrolyzable fraction which accounts for approximately 35 to 40 percent of the residual protein. It contains hydroxyproline and hexosamine.

b. A trypsin resistant fraction which is rapidly hydrolyzed by collagenase and may be slowly attacked by repeated trypsin, hyaluronidase, and drying treatments. This fraction is suggestive of collagen.

c. A residue resistant to the above enzyme treatments. It contains amino acids but no proline or hydroxyproline.

University of California Medical Center (22).

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Discussion

Dr. Zacharias Dische (New York): I would like to say a few words concerning Dr. McEwen's very interesting paper, because I think the criticism by Dr. Balazs, while it is completely justified as far as the loose use of the words "residual protein" is concerned, may do a certain injustice to the paper and the results Dr. McEwen has shown.

It is perfectly true that the residual protein is a mixture which could be brought about by contamination, but in addition I think we must not dismiss the idea that it is a mixture by structural co-ordination of various things. It is difficult to explain Dr. McEwen's results only by simple combination, because almost 40 percent of the total protein goes off on the trypsin digestion.

One possibility to reconcile that with our concepts of the chemical nature of collagen is that in this residual protein there is a structural co-ordination of certain small collagen fibers by very small amounts of highly active high polymers of the kind of hyaluronic acid. As soon as the latter are removed, the structure disintegrates and perhaps goes simply into solution. I am not quite sure how Dr. McEwen determined the tryptic effects on his material. Perhaps this would be a crucial thing to answer.

We found in our residual protein, which was taken from the center of the vitreous, that we cannot remove certain traces of hyaluronic acid even by the most exhaustive washing and combined homogenization and treatment with hyaluronidase. We had the impression that there may be a structural combination between certain collagen fibers and hyaluronic acid which may be of major significance.

ENZYMATIC ACTIVITIES OF THE LENS*

A PRELIMINARY SURVEY

BERNARD WORTMAN, PH.D., AND BERNARD BECKER, M.D.
Saint Louis, Missouri

INTRODUCTION

The introduction of sensitive analytical methods and improved techniques for obtaining microquantities of tissue[1, 2] makes it possible to study the enzymatic patterns of parts of the lens. This report represents a preliminary investigation of the quantitative distribution of certain enzymes in this organ. For this study representative enzymes of the glycolytic cycle (aldolase, glucose-6-phosphate isomerase, and lactic dehydrogenase), as well as oxidative pathways, such as the citric acid cycle (fumarase and malic dehydrogenase) and the hexose monophosphate shunt (glucose-6-phosphate dehydrogenase), were selected for measurement.

MATERIALS AND METHODS

Eyes from adult rabbits were enucleated and the globes opened from the posterior aspect. Lenses were removed and placed on moist filter paper, chilled by an iced glass-stage. Excess humors were carefully removed by blotting with filter paper. Anterior and posterior capsules were separated from the remaining cortex and nucleus by incising along the equator. The three portions were separately weighed and homogenized in a bovine plasma albumin solution (0.05 percent in water) to the appropriate dilution.

Enzymatic activities were determined after incubation of one to 50 micrograms of tissue in substrate reagent volumes of 10 to 50 microliters for 30 or 60 minutes at 37°C. The product formed from a substrate or from a cofactor was measured by fluorimetry or spectrophotometry. Substrate reagents and products measured are summarized in Table 1. The details of the biochemical methods and instrumentation are thoroughly discussed and published elsewhere.[3-5]

RESULTS

The enzymatic activities of each isolated tissue of lens, expressed as moles of product formed per kilogram wet tissue per hour at 37°C., are summarized in Table 2. It is observed from these data that malic and lactic dehydrogenases were approximately three times higher in lens substance proper than in either anterior or posterior capsule. Although these two enzymes demonstrated different levels of activity, no differences in either were noted on comparing anterior and posterior capsules. The activities of aldolase, glucose-6-phosphate dehydrogenase, and glucose-6-phosphate isomerase were approximately 1.5 to 2.0 times higher in anterior than in posterior capsule. Compared to the capsule, combined cortex and nucleus showed about two times as much aldolase and glucose-6-phosphate isomerase activity. Glucose-6-phosphate dehydrogenase was the only enzyme studied that was noted to have the least activity in the lens substance proper. Fumarase was observed to have the same level of activity in all parts of the lens assayed.

DISCUSSION

The use of micro methods increases the sensitivity sufficiently to detect enzyme activities in small numbers of cells. It would be of interest to compare values obtained by these microchemical methods with those reported previously.[6, 8] Pirie et al.[9] and Van Heyningen et al.[10] have made measurements of citric acid cycle and glycolytic enzymes as

* From the Department of Ophthalmology and the Oscar Johnson Institute, Washington University School of Medicine. This study was conducted in the laboratories of the Department of Pharmacology where facilities, technical aid, and invaluable advice were generously provided by Dr. O. H. Lowry and associates.

TABLE 1

SUBSTRATE REAGENTS

Enzyme	Aldolase	Glucose-6-PO_4 Isomerase	Lactic Dehydrogenase	Glucose-6-PO_4 Dehydrogenase	Fumarase	Malic Dehydrogenase
pH	8.7	8.2	7.4	8.9	6.8	8.4
Buffer	Hydrazine	TRIS	TRIS	AMP_2	Phosphate	TRIS
Cofactor	None	None	DPN.H	TPN + Mg. + + EDTA	None	DPN.H
Substrate	Fructose-1, 6-di PO_4	Glucose-6-PO_4	Pyruvic acid	Glucose-6-PO_4	Fumaric acid	Oxalacetic acid
Product measured	Glyceraldehyde-3-PO_4	Fructose-6-PO_4	DPN +	TPN.H	Malic acid	DPN +

AMP_2 = 2-amino-2-methyl-1, 3-propandiol.
DPN = diphosphopyridine nucleotide.
EDTA = ethylene diamine tetra acetate.
TPN = triphosphopyridine nucleotide.
TRIS = tris. (hydroxy-methyl)-amino-methane.
Mg. = magnesium.

well as glutathione and cytochrome reductases, and Kinoshita[11] measured glucose-6-phosphate dehydrogenase in whole lens homogenate. However, from the published data, it was not possible to make precise comparisons. Kinoshita's suggestion that glucose-6-phosphate dehydrogenase is highest in anterior capsule is quantitatively demonstrated by these data.

Kinoshita[11] reported that for every three molecules of glucose metabolized via glycolysis, one is metabolized via the hexose monophosphate shunt. However, Dische and Ehrlich[12] did not find the utilization or accumulation of shunt intermediates by lens capsule. Under the conditions of this investigation, it was found that glucose-6-phosphate isomerase was over 200 times more active than glucose-6-phosphate dehydrogenase in lens substance proper and about 40 times as active in capsule. Although Kinoshita concluded that pyruvic acid is not metabolized by the lens, the presence of an active lactic dehydrogenase as well as citric acid cycle

TABLE 2

ENZYMATIC ACTIVITIES*

	Aldolase	Glucose-6-PO_4 Isomerase	Lactic Dehydrogenase	Glucose-6-PO_4 Dehydrogenase	Fumarase	Malic Dehydrogenase
Cortex and Nucleus	0.38 ±0.03	9.66 ±0.13	4.13 ±0.04	0.04 ±0.001	0.26 ±0.02	2.59 ±0.02
Anterior capsule	0.16 ±0.03	4.44 ±0.18	1.32 ±0.02	0.10 ±0.005	0.22 ±0.02	0.98 ±0.01
Posterior capsule	0.10 ±0.01	2.31 ±0.01	1.31 ±0.01	0.06 ±0.005	0.21 ±0.03	1.00 ±0.004
Number of analyses	9	9	3	12[a,b]	6[c]	3

* Mean ± standard error in moles of product formed/kg. wet tissue/hour at 37°C.
a = 18 for anterior capsule.
b = 15 for posterior capsule.
c = 3 for posterior capsule.

enzymes demonstrates the capacity for metabolizing pyruvic acid. The citric-acid cycle enzymes, fumarase and malic dehydrogenase, were not as active as lactic dehydrogenase, but they were each many times more active than glucose-6-phosphate dehydrogenase. These findings suggest greater activity of the citric acid cycle than of the hexose monophosphate shunt.

The differences between the present data and those reported may be related in part to discrepancies between in vivo and in vitro studies. The possibility remains that in situ some enzymes may function under less favorable conditions as to such limiting factors as insufficient substrate, product accumulation, or nonoptimal conditions of pH.

Lactic acid is the last product formed in glycolysis, and so lactic dehydrogenase may represent glycolytic metabolism. Similarly, if one used the malic dehydrogenase data to represent the citric acid cycle and glucose-6-phosphate dehydrogenase to represent the hexose monophosphate shunt, comparisons of the capacity of the various pathways are possible. It is suggested by the data that lens cortex and nucleus are more glycolytic dependent than is the capsule. Furthermore, it appears that all parts of the lens are predominantly glycolytic and are capable of deriving more energy from the citric acid cycle than from the hexose monophosphate shunt. The question of how effectively this potential capacity is utilized in vivo cannot be answered from the present study.

Since no attempt was made to separate the epithelium from the anterior capsule, the greater activity demonstrated relative to the posterior capsule could be accredited to epithelial cells. However, the similarities in activities of malic and lactic dehydrogenases in anterior and in posterior capsules suggest that there is not sufficient epithelial tissue present to have influenced the results. It appears entirely possible that some enzymes are in greater concentration in anterior than in posterior capsule.

Summary

Quantitative measurements of aldolase, glucose-6-phosphate isomerase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase, fumarase, and malic dehydrogenase are reported for the cortex and nucleus, anterior capsule, and posterior capsule of rabbit lenses. The data demonstrate differences in activities of various enzymes in different parts of the lens. The higher activities found for glycolytic and citric acid cycle enzymes than for glucose-6-phosphate dehydrogenase fail to support reports of the significance of the hexose monophosphate shunt in the metabolism of lens.

640 South Kingshighway (10).

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Discussion

Dr. Jin H. Kinoshita: The new and remarkable technique employed by the authors should prove extremely useful in demonstrating the types of enzymes present in ocular tissues which we know so little about. The ultramicro analyses should now make possible the enzyme assays of the lens epithelium and corneal endothelium.

I would like to comment on certain aspects of their interpretations. From the paper just given, one is left with the impression that since the hexose phosphate isomerase is so much more active than the G-6-P dehydrogenase, 200 times, any glucose-6-phosphate formed would be very quickly channeled into the glycolytic pathway and very little would be directed into the shunt mechanism. However, the action of the isomerase, like any enzyme, is to accelerate the attainment of an equilibrium of the reaction and does not change or shift the extent of such an equilibrium. In this reaction, at equilibrium, Lohmann has shown that 70 percent of the hexose would be in the form of glucose-6-phosphate and 30 percent in the form of fructose-6-phosphate. The very active isomerase enzyme found in lens indicates, therefore, that when glucose-6-phosphate is formed there would be a rapid equilibration, in which some of it is converted to fructose-6-phosphate but most of it remaining as glucose-6-phosphate. Therefore the high isomerase activity does in no way determine the extent of the two pathways in question.

Another argument which might be presented to point out that the activity of the isomerase should not be used to compare the relative activities of the shunt and glycolytic pathways is that the isomerase is not only an enzyme of the glycolytic pathway but also part of the shunt mechanism.

For these reasons, another enzyme should be selected which would more appropriately represent the activity of the glycolytic mechanism. In a series of reversible reactions, which make up a metabolic pathway, the over-all rate of the pathway is not determined by the most rapid reaction but rather by the slowest of the reactions—that is, the rate limiting step. As shown by Olson the rate-limiting step in the glycolytic mechanism of liver is the reaction catalyzed by phosphofructokinase. It might well be that this is also the case in the lens. In the absence of any information concerning the activity of this enzyme in lens, let us use the activity of aldolase, since it appears to be the least active of the enzymes so far measured, as more closely representing the rate determining step of the glycolytic mechanism. The ratios of aldolase activity to G-6-P dehydrogenase activity is 10/1 in the cortex-nucleus section and only 1.6/1 in both the anterior and posterior capsules. These comparisons then place the shunt mechanism in a more favorable light and become more consistent with our estimations that in intact lens one out of every five molecules of glucose utilized is metabolized via the shunt mechanism.

However, a more basic question is whether one can reconstruct the complex pattern of glucose metabolism of completing pathways from the measurements of enzyme activities under optimal conditions. In this study, enzyme activities were measured in homogenates, at pHs not normally found in lens, in a foreign environmental fluid, and in presence of excess substrate. This is the usual procedure of the enzyme chemist who chooses the condition for the test to give the maximum rate of reaction. Can these enzymatic activities be used as an index as what is happening in the intact lens? To see if this question can be answered let us compare the activities of the enzymes observed in homogenates and in the intact lens. The authors found that the dehydrogenase activity is 40 μM/gm./hr. Transposing our data observed in intact lens to the same units, we find that only 0.2 μM/gm./hr. of the hexose is acted on by the dehydrogenase. This indicates that only 0.5 percent of the total capacity of the enzyme is utilized. Similarly, in the glycolytic pathway, the aldolase activity shows that 380 μM/gm./hr. of hexose can be metabolized by this pathway. In the intact lens the extent of glycolysis is that one μM/gm./hr. of glucose is metabolized. Here again only a fraction of a percent of the total enzyme capacity is utilized. Since, in the observations made in homogenates, we are not duplicating the level of enzyme activity normally found in the intact lens it may be difficult to assess the relative activities of competing pathways from such observations.

In the intact tissues other factors undoubtedly come into play. Included in these factors, many of which little is known, would be the regulating mechanisms, the substrate concentrations, the environmental conditions, the spatial localization of enzymes, and the availability of coenzymes. Many of these factors are probably important in explaining why the citric acid cycle appears to be sluggish in the intact lens. The enzymes of the TCA cycle are undoubtedly present in lens as first shown in 1951 by Ely and the activities of two of these enzymes have been more precisely measured in the present work. Regardless of how active these en-

zymes may appear to be unless the electron transmitting system is also functioning. The citric acid cycle may not operate very effectively.

DR. BERNARD WORTMAN (closing): Thank you, Dr. Kinoshita. There was much weight in your comments.

We attempted to be cautious and not to draw any conclusions about what may be occurring in vivo. This study is concerned with the measurement of amount of enzyme present in parts of the lens and will lead into the quantitative histochemistry of the eye. Enzymes may be demonstrated in homogenates. If whole cells are used, there is the possibility that substrates may not enter and reach the activating sites, and the results may be misleading.

If the use of optimal conditions of pH and substrate raises questions, let me say that if we may measure maximal amount of enzymatic activity, we may have an index of the amount of enzyme present. A satisfactory intracellular pH measurement has never been made. It is possible that enzymes may function at their optimal pH in vivo, just as it is possible that under more "physiological conditions" the enzymatic activity may be less than optimal.

All one may conclude from our study is the practicality of some microprocedures which have been used to show that certain enzymatic capacities are present in parts of the lens.

EFFECT OF AGE UPON LENS METABOLISM*

I. PRELIMINARY OBSERVATIONS

HARRY GREEN, PH.D., AND SHIRLEY A. SOLOMON, B.S.
Philadelphia, Pennsylvania

Perhaps the most common type of ocular ailment resulting in diminution of visual acuity in older persons is senile cataract. Many theories have been advanced but relatively little is known concerning the mechanism of pathogenesis of cataract. The lens is considered to grow to maturity and then to undergo physicochemical changes associated with senescence. These include changes in permeability of the capsule, chemical composition, metabolic activity, and size and weight which may manifest themselves in alterations of function and visible degenerative disturbances.

Our present state of knowledge, however, does not permit us to assign to any of these changes the role of causative or responsible factor in the development of cataract. On the other hand, most investigators and theoreticians in this field favor the concept that the growth, function, and maintenance of transparency of the lens are fundamentally dependent upon the active metabolism of the tissue.

Reports in the literature have suggested that the metabolic activity of the lens decreases with increasing weight or age of that organ. Lenses with incipient or advanced cataract showed a marked diminution in metabolic activity.

Müller[1] observed that the ability of excised bovine lens to consume glucose from Ringer's solution covered with paraffin decreased with increasing age of the animal. On the basis of the amount of inorganic phosphate disappearing from the medium and the amount of organic phosphate formed by intact excised lenses, cut-up lenses, and cell-free extracts maintained for five hours at 37°C., Süllmann[2] suggested that the ability to metabolize glucose was greater for calf lenses than for beef lenses. Lenses with incipient or advanced cataract showed a marked decrease in the ability to form organic phosphate esters.

At about the same time Müller[3] found that the ability of saline extracts of cattle lens to form alkali-saponifiable phosphate (triosephosphate) from fructose diphosphate decreased with age of the animal.

* From the Department of Research, Wills Eye Hospital, and the Department of Physiological Chemistry, Graduate School of Medicine, University of Pennsylvania. This investigation was supported, in part, by a research grant, B-693(C), from the National Institute of Neurological Diseases and Blindness, of the National Institutes of Health, United States Public Health Service.

More recently Nordmann and Mandel[4] reported that the concentration of easily hydrolyzable phosphate (adenosine tri- and diphosphate) and of creatine phosphate in lenses of cattle, rabbits, and rats declined with the age of the specie studied. Subsequently Müller and co-workers[5] found, in agreement with Nordmann and Mandel, that the concentration of seven-minute phosphorus was higher in younger rabbits than in older ones.

Müller and Kleifeld[6, 7] also found that young rabbit lenses weighing between 200 and 350 mg. when incubated at 37°C., with glucose and a buffered solution containing $P^{32}O_4$ incorporated 50 percent more P^{32} into organic phosphate (seven-minute and difficultly hydrolyzable phosphorus) than did older lenses weighing between 450 and 650 mg. under similar conditions.

From these observations it might be inferred that the metabolic activity of the lens, measured in terms of glucolysis, decreases with increasing weight or age of the lens. In this connection it is important to note that the results and conclusions of the foregoing investigators are based upon the comparative evaluation of equal weights of younger and older lenses. In practically all cases the older lens weighed from 40 to 100 percent more than the younger lens, and the measured criterion of activity associated with the younger lens was not more than twice as much as that with the older lens. It is obvious, therefore, that when the results are evaluated in terms of the whole lens, either there is little difference in metabolic activity between the two lens groups or the older lens shows the greater capacity.

With the elucidation of the pathway of anaerobic carbohydrate metabolism in the crystalline lens by Green and co-workers,[8] it became possible to study the effect of age upon the individual intermediary reactions. Since metabolism is a regular sequence of enzymatically controlled reactions, a disturbance of any one of these could lead to a slowing-down of or interference with the formation of the end-product, lactic acid, and result in a decrease in metabolic activity of the lens. It was reasonable to expect, therefore, that if such a slowing down of lens metabolism occurred with increased age of the animal, comparative studies with young and older lenses might indicate the point in the sequence of reactions at which the enzymatic mechanism was affected.

This is a report of a preliminary study of the problem. The complete details and results will be published later.

Procedure

Extracts of rabbit lenses were incubated at 37°C., usually for 60 minutes, in the presence of suitable additions of necessary co-factors and varying concentrations of substrate. Glucose, glucose-6-phosphate, and fructose diphosphate were the substrates used. The substrate concentration above which there was no appreciable increase in the yield of lactic acid is referred to as the optimal concentration. The amount of lactic acid produced under the optimal conditions of substrate and co-factor concentrations is a measure of the functional capacity of the lens extract.

Young lenses were obtained from pigmented rabbits three to four weeks of age and weighed on the average 140 mg. Old lenses came from rabbits three years of age and weighed on the average 600 mg. Each experiment was performed with the extract equivalent to one lens and the results are evaluated in terms of lactic acid produced per lens equivalent, per mg. wet weight and per mg. protein.

Results

The results to date suggest that the functional capacity of the older lens to produce lactic acid exceeds that of the younger lens by 50 to 75 percent. It should be noted that these studies are being done with cell-free extracts of lenses whose structural integrity was destroyed. In an intact tissue such factors as the spatial location of the enzymes,

pH or thermodynamic conditions, low apoenzyme concentration, coenzyme or activator deficiency, permeability, and the presence of inhibitors serve to control the functional metabolic activity of the system.

In cell-free extracts, however, fortified with additions of the various necessary coenzymes and activators and maintained at a given pH, the most important factor becomes the relative concentration of the individual enzymes in the multi-enzyme system (and, perhaps, the nature of the enzymes). Consequently, the observed differences in the functional capacities of the two lens-age groups may be ascribed to the quantitative difference in the amount of enzyme activity per lens.

Accordingly, it is suggested that in the rabbits studied, the increase in functional capacity of the older lens to metabolize carbohydrate anaerobically is associated with an increase in the enzyme content per lens. It is interesting to note, moreover, that although the functional capacity of the lens increased 50 to 75 percent, the weight of the lens increased from an average of 140 mg. to 600 mg., an increase of more than 300 percent, and the protein content showed a similar increase.

Thus, despite an increase in the total amount of enzyme activity, there occurs a marked decrease in the enzyme concentration; that is, the amount of activity per unit weight or per mg. protein. Whether this progressive decrease in enzyme concentration of the lens is responsible for the decreased rate of formation of new lens fibers with increased age, and leads to lens changes characteristic of senescence, remains for study.

1601 Spring Garden Street (30).

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Discussion

Dr. P. J. Leinfelder (Iowa City, Iowa): Dr. Green has utilized the enzymologic technique of cell-free lens extracts in order to quantitate the glycolytic reactions in the lens. Previous work by us and others has shown that quantitation of glycolysis was extremely difficult if whole lenses were used, because variations in individual measurements as high as 300 percent were obtained. Although the data suggested that there was a greater glycolysis in the young lens than in the older on a per milligram wet weight basis, we felt that it was unjustifiable to make specific quantitative conclusions from the data.

Dr. Green feels that by this method the reaction goes to an end-point, and that he can obtain repetitive results. He has utilized the whole lens unit as an index, although he has also presented the data on the basis of wet weight of the lens. I do not believe one can criticize him in desiring to use the whole lens, because all of us believe that the lens is

a metabolic unit. Although the whole lens unit is unconventional, I think none of us can say that the wet or dry weight represents a more accurate index, for there are errors in these methods—the inherent error due to the fact that the lens loses water as it grows older. With any unit of reference we must consider whether there is inert tissue in the lens. If there is inert tissue, the whole lens is a better unit.

Metabolism of the lens is important in order to maintain the lens but in addition energy is needed to provide for growth, for maintenance of transparency, and for active transport of ions and metabolites. We know that the young lens grows more rapidly. This requires more energy for growth and, of course, with this there would be added energy requirement for active transport of ions, metabolites, and water. As the lens grows older, these processes would slow down because of slower growth. However, I think it would be erroneous to conclude that the slowing down of metabolic processes because of physiologic change would be the cause of cataract.

DR. HARRY GREEN (closing): I should like to thank Dr. Leinfelder for his comments and observations and for his discussion of the general problems relative to this study. Of fundamental importance is the question whether the development of senile cataract is an inevitable result or a pathologic deviation of the normal physiologic aging processes of the lens.

OXIDATIVE PHOSPHORYLATION BY CILIARY PROCESSES*

ELMER J. BALLINTINE, M.D., AND MORTON WAITZMAN, PH.D.
Cleveland, Ohio

It has been demonstrated that certain basic cyanine and styrylquinoline dyes inhibited oxidation by molecular oxygen in respiring tissue homogenates[2] and a purified cytochrome oxidase preparation[3] presumably at the cytochrome oxidase step. These dyes were found to concentrate in ciliary epithelium when administered to rabbits by injection into the internal carotid artery, and, when administered in appropriate concentration, there was a reversible reduction in intraocular pressure, aqueous humor bicarbonate, and aqueous humor ascorbic acid without reduction in oxygen consumption in the isolated ciliary processes.[1]

These experiments indicated that the dyes were interfering with aqueous humor formation at some site other than the oxidation by molecular oxygen. Subsequently, it was shown that in respiring tissue homogenates the dyes in lower concentrations than those which inhibited oxygen consumption uncoupled oxidation from phosphorylation without marked reduction in oxygen uptake.[8]

It was presumed that the dyes might be acting in the ciliary body on oxidative phosphorylation to reduce aqueous humor formation and that oxidative phosphorylation might be an essential step in formation of aqueous humor. The following experiments were undertaken, therefore, to determine if the ciliary processes are the site of oxidative phosphorylation and to estimate, if possible, the extent and efficiency of the system.

MATERIAL AND METHODS†

Eyes from albino rabbits or pigmented hogs were obtained from freshly slaughtered animals and placed in chipped ice for about

* From Western Reserve University, School of Medicine, and The University Hospitals of Cleveland. This study was supported in part by Research Grant B-121 from The National Institute of Neurologic Diseases and Blindness, National Institutes of Health.

† THE FOLLOWING ABBREVIATIONS ARE USED
ATP = APPP = Adenosine triphosphate.
ADP = APP = Adenosine diphosphate.
AMP = AP = Adenosine monophosphate.
P = Orthophosphate.
^{32}P = Orthophosphate labeled with phosphorus 32.
I = Inosine monophosphate.
TCA = Trichloroacetic acid.
Dye 263 = 1'-ethyl,-3,6-dimethyl-2-phenyl-4-pyrimido-2'-cyanine chloride.
"Pigmented Processes" = Homogenate of ciliary processes from normally pigmented hog eyes.
"Albino Processes" = Homogenate of ciliary processes from albino rabbit eyes.

1.5 hours until they could be transported to the laboratory for further dissection. In the laboratory, the eyes were opened equatorially and everted over a specially constructed expanding holder in a bath of iced Krebs-Ringer solution[5] (medium III, omit calcium chloride, phosphates, and organic compounds). Under a binocular dissection microscope, 10 to 20 mg. of individual ciliary processes per albino rabbit eye were dissected from their attachments to the iris and kept in a moist chamber in melting ice. The ciliary processes from pigmented hog eyes could be dissected from the ciliary body and posterior surface of the iris in a continuous ribbon weighing between 50 and 100 mg. The tissue was homogenized in modified Potter-Elvehjem glass homogenizers in the presence of substrate and phosphate-buffered Krebs-Ringer solution so that a five to eight percent suspension of tissue resulted.

In a 5.0 ml. Warburg vessel, 0.7 ml. of this suspension was placed in the main chamber, and the tetrasodium salt of adenosine triphosphate (ATP), neutralized to pH 7.4 with HCl, and carrier-free ^{32}P labeled orthophosphoric acid, having a total activity of about 2,000,000 disintegrations per minute, were placed in the side-arm. The center well contained 6 N KOH. Vessels were placed on manometers, immersed in a constant temperature bath at 20°C., and shaken at about 120 oscillations per minute. After gassing and equilibration, the manometers were closed, the side-arm contents tipped into the body of the vessel, and oxygen consumption recorded. The final composition of the reaction mixture was: succinate, 0.02 M; ATP, 1,750 μM; 0.25 (± 10 percent) microcuries of ^{32}P; $MgCl_2$, 1.11×10^{-3} M; Na_2HPO_4, 3,500 μM, and this buffer adjusted to pH 7.4 with 1 N HCl; final solution pH 6.8-7.0; NaCl, 0.111 M; HCl, 0.005 M; Mg SO_4, 0.001 M. At the end of the incubation, the reaction was stopped by adding 0.1 ml. of 30 percent TCA.

The protein precipitate was separated by centrifugation, and the phosphates in the supernatant were separated by two solvent paper chromatography described by Eggleston and Hems[6] and later modified by Krebs and Hems.[7]* The papers were dried at 80°C. for seven minutes, thus hydrolyzing the gamma and the beta phosphate of the nucleotides.

Under ultraviolet light the areas of nucleotide-quenched fluorescence were outlined lightly in pencil. The papers were then sprayed with a perchloric acid-ammonium molybdate solution and exposed to an atmosphere of hydrogen sulfide. The areas containing free phosphate ions were visible as blue phospho-molybdate complex. These areas were cut out and ashed with a 1:1 mixture of 10 N perchloric acid and 10 N sulfuric acid. The total phosphate in the ashed residue of the fractions was determined by a modification of the method of Berenblum and Chain.[2] An aliquot of the benzene-butanol phase containing the phospho-molybdate complex was pipetted into planchets and the rate of ^{32}P beta emission counted with the Tracerlab "Autoscaler" using a thin mica window Geiger-Mueller tube. The trichloroacetic acid precipitates from the Warburg vessels were washed five times with glass-distilled water, then ashed with the perchloric acid-sulfuric acid mixture, and the phosphate content and rate of ^{32}P disintegration determined as described above.

Using the same methods for protein precipitation and phosphate separation, the endogenous phosphates of homogenates of ciliary processes were determined.

The incubation studies were checked by duplicating the experiment of Krebs and Hems[6] with homogenates of rabbit liver and kidney cortex (3.3 percent concentration of tissue in reaction mixture). Dye No. 863

* In distilling the isopropyl ether for the chromatography solution, it is important to be certain that the crude isopropyl ether is free of peroxide and that solid ferrous sulfate be kept in the distilling flask to insure that no peroxides will form during the distillation. Vapors of isopropyl peroxides may decompose explosively when present in sufficient concentration.

1×10^{-3} M was included in some of the reaction mixtures.

Results

ATP, ADP, AMP, and orthophosphate were identified in ciliary processes by comparing their chromotographic migration with pure samples, by chromotographing mixtures of pure samples with the unknowns using different solvents, and by spectrophotometry of the chromatographically separated spots on paper. The results are summarized in Table 1.

Inosine phosphate appeared in the reaction mixtures. The conditions responsible for the accumulation of this substance and the details of its identification will be presented in another communication.

The results from one experiment with "pigmented processes" and one experiment with "albino processes" are shown in Table 2 and Table 3. The results in anaerobic experiments and with addition of dye No. 863 are shown in Tables 2A and 3A. All concentrations are expressed as micromoles in the reaction mixture. All activities are expressed as percent of total radioactivity in the reaction mixture. All specific activities

TABLE 1

ENDOGENOUS PHOSPHORUS IN CILIARY PROCESSES
(All values expressed in μM/gm. tissue)

	Pigmented Hog Eyes				Nonpigmented Rabbit Eyes			
	A+D*		A†		A+D*	A†		
Experiment #	20	22‡	20	22	18‡	2	7	18
Total "Organic" P†			2.96	1.53		2.97	2.36	3.69
ATP	0.60	0.0$_x$			0.15			
ADP	0.55	0.0$_x$			0.22			
AMP	0.72	0.0$_x$			0.29			
Ortho-P	3.77	3.74	3.77	3.74	3.27	4.03	?	3.27
Total	5.64	—	6.73	5.27	3.93	7.00	—	6.96
TCA Supernatant	8.68	5.82	8.68	5.82	7.02	5.47	6.58	7.02
Washed TCA Residue	26.43	17.71	26.43	17.71	29.21	23.84	17.10	29.21

* Ascending plus descending chromatography.
† Ascending chromatography only. Represents total of slow moving P components.
‡ In these experiments inosine nucleotides are shown to be absent.
$_x$ Trace present: Sample too small for measurement.

TABLE 2

PIGMENTED PROCESSES

		Aerobic						Anaerobic			
Time (min.)		0	1/2	6	10	15	20	4	10	15	20
ATP	Micromols	1.78	1.54	1.15	1.30	1.54	1.51	1.46	1.22		1.12
	Activity	0.0	0.8	6.8	7.9	11.3	12.5	3.22	3.02	2.54	4.23
	Sp. activity	0.0	0.5	5.9	6.1	7.3	8.3	2.20	2.5	2.6	3.8
ADP	Micromols	0.20	0.24	0.24	0.16	0.16	0.20	0.36		0.36	0.48
	Activity	0.0	0.05	0.31	0.4	0.4	0.4				
	Sp. activity	0.0	0.21	1.29	2.5	2.5	2.0				
AMP	Micromols		0.08		0.08			0.16		0.08	0.24
Inosine	Micromols					0.24	0.16		0.40	0.32	0.48

O_2 consumption 0.076 micro-atoms/min./vessel.

TABLE 2A

PIGMENTED PROCESSES

Time (min.)		20				25			
Conditions		Aerobic	Anaerobic	Aerobic + Dye 863	Anaerobic + Dye 863	Aerobic	Anaerobic	Aerobic + Dye 863	Anaerobic + Dye 863
ATP	Micromoles	1.48	0.82	1.32	1.27	1.48	1.00	1.24	0.82
	Activity	19.8	4.05	0.72	0.72	29.4	4.6	0.9	0.9
	Sp. activity	13.3	5.0	0.5	0.6	13.8	4.6	0.7	1.1
ADP	Micromoles		0.44	0.72	0.48	0.20	0.76	0.52	0.44
	Activity		0.8	0.13	0.12	0.9	0.8	0.1	0.2
	Sp. activity		1.8	0.2	0.25	4.3	1.0	0.2	0.4

O_2 consumption: micro-atoms/min./vessel.
Aerobic 0.045.
Aerobic + Dye 0.003.

TABLE 3

ALBINO PROCESSES: AEROBIC

Time (min.)		0	1	4	6	10	15	20	20
ATP	Micromols	1.54	1.16		1.02	0.68	0.23	0.22	0.16
	Activity	0.54	2.13		4.21	3.52	3.59	3.46	3.13
	Specific activity	0.35	1.24		4.13	5.18	15.6	15.7	19.6
ADP	Micromols	0.08	0.46	0.71	0.78	0.62	0.54	0.44	0.44
	Activity	0.0	0.03		0.24	1.82	2.53	2.86	3.03
	Specific activity	0.0	0.06		0.31	2.94	4.68	6.50	6.89
AMP	Micromols	0.08	0.16	0.37	0.52	0.52	0.68	0.76	0.68
	Activity	0.0			0.03	0.06	0.03	0.09	0.15
	Specific activity	0.0			0.06	0.12	0.08	0.12	0.22
Ortho-phosphate	Micromols	3.80	4.00	5.37	5.36	5.76	6.28	6.48	6.32
	Activity	99.46	97.8	96.	95.50	94.6	93.5	93.3	93.
	Specific activity	26.17	24.5	18.	17.8	17.4	14.9	14.4	14.7
Inosine	Micromols					0.28	0.40	0.56	0.68
	Activity						0.36	0.24	0.76
	Specific activity						0.90	0.43	1.12

Average O_2 consumption 0.05 micro-atoms/min./vessel.

TABLE 3A

ALBINO PROCESSES

Elapsed Time (min.)		0	5	10			20		
Conditions			Anaerobic	Anaerobic	Anaerobic †Dye 863	*Aerobic †Dye 863	Anaerobic	Anaerobic †Dye 863	*Aerobic †Dye 863
ATP	Micromols	1.56		0.53	0.26	0.26	0.21	0.0	0.0
	Activity	0.0	0.66	0.76	0.40	0.22	0.38	0.0	0.0
	Sp. activity	0.0		1.40	1.50	1.2	1.8	0.0	0.0
ADP	Micromols	0.0	0.60	0.26	0.7	0.56	0.12	0.08	0.24
	Activity		0.13	0.40	0.06	0.08	0.10	0.10	0.08
	Sp. activity								
AMP	Micromols	0.0	0.16	0.64	0.64	0.48	0.96	0.80	1.04
	Activity	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Sp. activity	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Inosine	Micromols	0.0	0.0	0.36	0.13	0.08	0.48	0.48	0.0
	Activity			0.22	0.25	0.04	0.14	0.1	0.0
	Sp. activity					0.5	0.3	0.25	0.0

*Aerobic †Dye 863 – O_2 consumption 0.02 micro-atoms/min./vessel.

are expressed as percent of radioactivity in the reaction mixture per micromole adenosine and/or inosine nucleus.

In Figures 1 and 2 the results from three aerobic experiments with "albino processes" are plotted. The smooth curves were derived by adjusting the analytic results so that the sums of the micromoles of phosphate in each fraction and the sum of the micromoles of purine nuclei in each fraction at each time are constants equal to the average total phosphate and average total purine, respectively.

Figure 3 shows the incorporation of ^{32}P into nucleotides in reaction mixtures with pigmented processes in two experiments.

Figures 4 and 5 show the results of determinations of incorporation of ^{32}P into the TCA precipitate from the reaction mixtures.

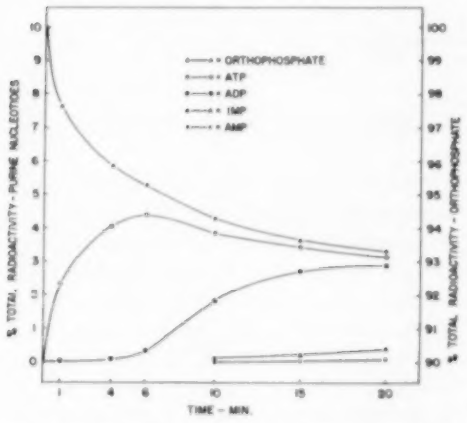

Fig. 1 (Ballintine and Waitzman). The results from three aerobic experiments with "albino processes."

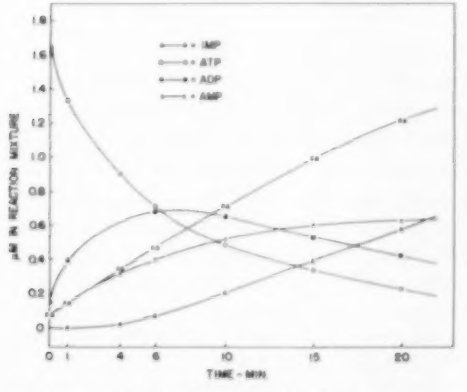

Fig. 2 (Ballintine and Waitzman). The results from three aerobic experiments with "albino processes."

Discussion

The presence of endogenous quantities of the three adenosine nucleotides in pigmented and nonpigmented ciliary processes is shown in Table 1. Calculations show that endogenous purine nucleus in the pigmented hog eye suspension will account for about two percent (half of which is AMP) of the total purine substance present in the reaction mixtures used in the respiration measurements. It is to be noted, also, in Table 1 that there is considerably more "total organic P" (slow moving component) in the "ascending only" analysis than there is in the total of the separated fractions in the ascending and descending chromatograms. The difference between these amounts is phosphate compounds which are further separated from the purine nucleotides during the descending chromatography.

Table 2 shows that the homogenates from pigmented hog eyes incorporate orthophosphate into ATP and that the rate of synthesis of ATP is rapid enough to prevent accumulation of ADP from the hydrolysis of ATP. The reactions may be summarized: $APP + {}^{32}P \underset{V2}{\overset{V1}{\rightleftarrows}} APP\,{}^{32}P$. Since the direct phosphorylation of AMP to ADP by orthophosphate has never been produced in mammalian tissues, the incorporation of labeled orthophosphate into the ADP indicates that there is also transfer of orthophosphate to the beta position of ATP by the myokinase reaction.

$$APP\,{}^{32}P + AP \underset{V4}{\overset{V3}{\leftrightarrows}} AP\,{}^{32}P + APP$$

$$AP\,{}^{32}PP + AP \underset{V4}{\overset{V3}{\rightleftarrows}} AP\,{}^{32}P + AP$$

Under anaerobic conditions there is in-

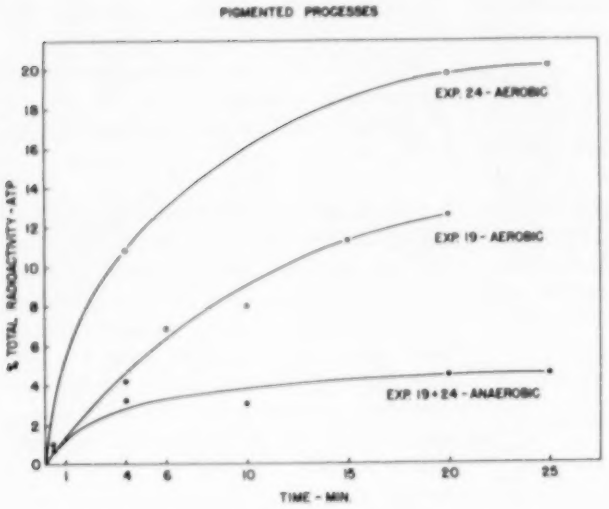

Fig. 3 (Ballintine and Waitzman). Shows the incorporation of ^{32}P into nucleotides in reaction mixtures with pigmented processes in two experiments.

corporation of labeled orthophosphate into ATP during the first four minutes of incubation at a rate almost equal to that for aerobic conditions. After the first four minutes the rate of anaerobic incorporation decreases rapidly while the rate of incorporation under aerobic conditions tends to be maintained for 20 minutes. Since the ratio of the specific activities of ADP to specific activity of ATP under both aerobic and anaerobic conditions is roughly constant (between three and four) the myokinase reaction is probably proceeding at about the same rate under both conditions. If the myokinase reactions were extremely rapid relative to the phosphorus incorporating reactions, the ratio of the specific activity of the ATP to the ADP should be two. Since the ratio is about 3.5, the myokinase reaction must be somewhat slower than the phosphorylating reaction.

If, under aerobic conditions, it is assumed that the increased rate of phosphate exchange is in addition to, rather than substituted for, the anaerobic exchange, a rough estimate of the minimum aerobic exchange can be obtained by subtracting the activity of the ATP under anaerobic conditions from the specific activity under aerobic conditions. This procedure ignores the loss of ^{32}P from the ATP by the reaction in the "V 2" direction.

Thus, from Experiment 19, the difference between the specific activity of the ATP in the aerobic and anaerobic experiment was 3.6 and 4.7 at end of 10 and 15 minutes, respectively. Since there were 1.5 moles of ATP in the reaction vessel, the net transfer of radioactivity into the ATP was 3.6×1.5 and 4.7×1.5. The average specific activity of the orthophosphate was 15 percent so that there was

$$\frac{3.6 \times 1.5}{15 \times 10} = 0.036 \text{ and } \frac{4.7 \times 1.5}{15 \times 15} = 0.030$$

micromoles of orthophosphate per minute transferred, ignoring the amount returned to the orthophosphate by phosphorolysis. In this experiment the O_2 consumption was 0.07 microatoms per minute so that the P:O ratio is not less than 0.5. Since each vessel contains about 0.038 gm. of tissue, this rate of O_2 consumption is about the same as that found previously in intact processes from albino rabbits[1] and in the same order as that found by deRoetth[10] for ciliary processes from beef eyes.

With homogenates of ciliary processes from albino rabbit eyes, ATP is not main-

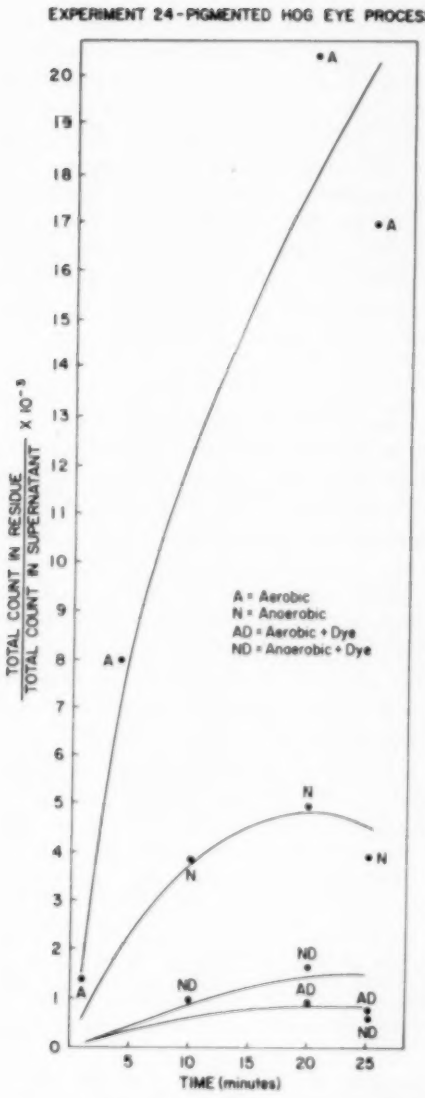
EXPERIMENT 24 - PIGMENTED HOG EYE PROCESSES
TOTAL COUNT IN RESIDUE / TOTAL COUNT IN SUPERNATANT X 10⁻³
A = Aerobic
N = Anaerobic
AD = Aerobic + Dye
ND = Anaerobic + Dye
TIME (minutes)

Fig. 4 (Ballintine and Waitzman). Shows the results of determinations of incorporation of ^{32}P into the TCA precipitate from the reaction mixtures.

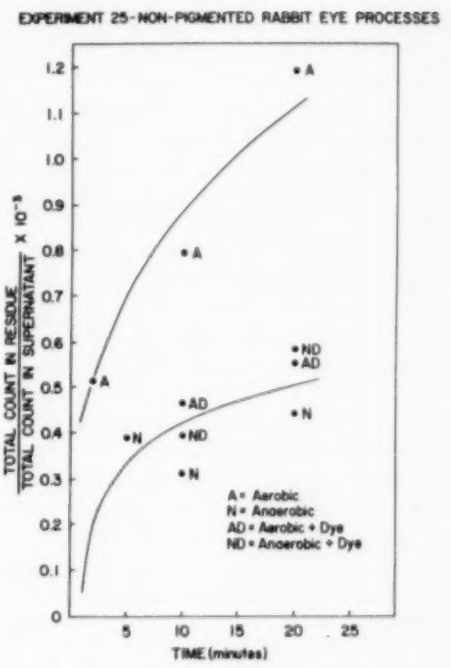
EXPERIMENT 25 - NON-PIGMENTED RABBIT EYE PROCESSES
TOTAL COUNT IN RESIDUE / TOTAL COUNT IN SUPERNATANT X 10⁻³
A = Aerobic
N = Anaerobic
AD = Aerobic + Dye
ND = Anaerobic + Dye
TIME (minutes)

Fig. 5 (Ballintine and Waitzman). Shows the results of determinations of incorporation of ^{32}P into the TCA precipitate from the reaction mixtures.

tained but undergoes rapid hydrolysis with accumulation of ADP initially, followed by accumulation of AMP and inosine (fig. 2). Anaerobically the ATP is still more poorly maintained and there is only slight exchange of ^{32}P with ATP. A rough calculation similar to that made for the "pigmented processes" shows that the net aerobic phosphorylation is not less than 0.03 micromoles of orthophosphate per minute and the P:O ratio is not less than 0.6.

In the anaerobic "pigmented process" experiments after 10 minutes, the concentration of ATP is maintained even though little ^{32}P is incorporated into the ATP while in the anaerobic albino process experiments there is rapid hydrolysis of ATP until none remains after 20 minutes. It appears, therefore, that the reason for the failure of the albino process homogenates to maintain ATP is the presence of high ATP-ase activity and not a relative deficiency in oxidative phosphorylation.

The labeling of ADP in the albino experiments occurs only after ^{32}P has been intro-

duced into ATP, indicating that the labeling of ATP is not the result of an exchange reaction proceeding through ADP.

In Table 3A ("albino processes") at 10 minutes the specific activity of the ATP in the vessels containing dye is about one-seventh that of the ATP of the aerobic vessel, and the anaerobic vessel is about the same as that of the aerobic vessel, while the oxygen consumption of the aerobic vessel containing dye is about 40 percent of the aerobic vessels. Apparently the dye prevents the aerobic incorporation of ^{32}P into ATP.

In Table 2A the concentration of ATP is maintained despite the presence of dye while the specific activity of the ATP in the vessels containing dye is one-twentieth that of the ATP of the aerobic vessels and one-seventh to one-fourth that of the ATP of the anaerobic vessels indicating that the dye prevents the aerobic and anaerobic incorporation of ^{32}P into ATP and does not produce its effects by accelerating the hydrolysis of ATP.

Figure 4 and Figure 5 show that the TCA precipitate from the reaction mixture with "pigmented processes" contains about 10 to 20 times as much ^{32}P as does the similarly treated material from the "albino processes." Under both aerobic and anaerobic conditions the addition of dye No. 863 to the reaction mixture reduces the amount incorporated by the "pigmented processes" residue to the same order as that of the "albino processes" residue but the presence of No. 863 in the "albino processes" reaction mixture did not reduce the amount incorporated below that observed under anaerobic conditions. The low concentrations of ATP in the later periods of the "albino processes" experiments does not account for the lower rates of incorporation because with the "pigmented processes" ATP concentrations were maintained despite the presence of dye.

Summary

1. Oxidative phosphorylation by homogenates of isolated ciliary processes from albino rabbits and normally pigmented hogs was demonstrated. The rates of oxygen consumption were comparable to those observed in intact ciliary processes. The P:O ratios observed were not less than 0.5.

2. Albino rabbit ciliary processes appear to contain a mechanism for hydrolysis of ATP which is much more active than the mechanism in pigmented hog eyes. Some details of this difference are presented.

3. Dye No. 863 prevented the incorporation of orthophosphate into ATP by these preparations and did not have a marked effect on the rate of hydrolysis of ATP.

10515 Carnegie Avenue (6).

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Discussion

Dr. Zacharias Dische (New York): I think the authors should be complimented for three things—for their courage in approaching a problem which is perhaps today the most involved in enzyme chemistry, namely, oxidative phosphorylation; for their very great skill in developing the right procedures to investigate a system where the limiting factor is the size (and this was done with great efficiency); and, finally, for their persistence to follow this very weary path of research.

The problem which was the main purpose of the investigation was to what extent the oxidative phosphorylation takes place in the ciliary processes, and whether the effect of the dye is really concentrated mainly on the oxidative phosphorylation. Here, of course, difficulties confronted the investigators, because they had to compromise in two respects due to the peculiar nature of their material.

To obtain a sufficient turnover in their samples they had to add succinic acid as a main substrate to their system, and in this way they succeeded indeed in obtaining a turnover which is comparable to the turnover in the intact ciliary body. The second thing was that they were not able to isolate the mitochondria and therefore had to operate on the whole homogenate.

This creates certain difficulties as far as transferring the results to the whole system is concerned, for the following reasons: The phosphorylation is not a single chemical reaction but is a complex system of probably four different reactions, and whether it takes place or not, and to what extent it takes place, depends on which reaction is the limiting reaction in the whole system; also, to a great extent, which substrate is used mostly in the oxidative reactions.

For example, when succinate is mainly oxidized, the efficiency of the oxidative phosphorylation is less than when other substrate, particularly alpha-keto-gluconate, is used. In this case we have a system which is driven by oxidation of succinic acid, and therefore the first question that arises is, what happens to the succinic acid? Is it oxidized to fumaric acid or does the oxidation proceed further? Depending upon the answer, the picture will be somewhat different.

The second factor is the use of homogenates and not of mitochondria. This introduces another difficulty. The peculiarities of the oxidative phosphorylation is that it depends in two different ways on structural integrity of the cell. The first is the structural integrity of these parts of the mitochondria themselves in which the reaction takes place, but in addition there is the relationship of the mitochondria to other intracellular factors which affect their metabolism; therefore, to obtain the maximum effectiveness of the oxidative phosphorylation we are forced to isolate the mitochondria not only from the cell but to liberate it from certain factors which under normal, fully physiologic conditions do not interfere but which do interfere when we take out the mitochondria under these unphysiologic conditions.

The effect with which we achieve these technical solutions of the problem shows up in the ratio P/O. The maximum ratio, as we see it today (and this still may be very doubtful concerning whether this is the final answer), is about 3:2. This can be obtained only when we operate with separated mitochondria under very specific conditions, namely, with hypotonic pretreatment of the mitochondria, which apparently liberates the mitochondria from certain ions which in some way are interfering with the metabolic efficiency of the system.

Dr. Ballintine and Dr. Waitzman obtained a P/O ratio of 0.5. This is a rather low ratio, and the interpretation of how far the effects of the dye on the oxidative phosphorylation can also be traced to the phosphorylating system as such must depend upon how far this low ratio is due to the relative decay of the whole system or to an accentuation of certain phases only of such a system due to the artificial conditions.

I would like to point out, however, that this investigation brought out certain highly suggestive and interesting facts. For example, this difference between the pigmented and nonpigmented ciliary body, which may show the way to interesting new investigations of this problem.

Dr. Elmer J. Ballintine: It certainly is true that the homogenate is extremely sensitive to the method of preparation, and we have done a few experiments to try to see if the small amounts of endogenous ATP in the cells themselves, that is, in the isolated ciliary processes, will incorporate ^{32}P from orthophosphate. These experiments might indicate whether this process is really occurring in the intact cell or not; but the amounts of endogenous ATP are small, and it taxes what we can do analytically.

The other obvious question is: How completely is succinate oxidized in this system?

We are now attempting to answer this question by determining the amounts of succinate, maleate, and furmarate remaining in the reaction mixture.

LENS METABOLISM WITH THE REVERSIBLE CATION SHIFT*

IV. The ability of various metabolites to replace glucose

Daniel J. Heinrichs,† M.D., and John E. Harris, M.D.
Portland, Oregon

Previous studies have indicated that the energy for active transport of cations across the lenticular barriers generally derives from the metabolism of glucose and to an undetermined extent requires oxygen.[1, 2] This is not strictly true of younger lenses which, under the conditions imposed by the experimental technique, may maintain cation transport for a period in a medium devoid of glucose.[3] (This is not observed if in addition oxygen is deprived). However, the results generally support the view that a continuous supply of glucose delivered via the aqueous is essential to the lens. Whether certain metabolites are capable of replacing glucose is not certain.

The present studies were undertaken to determine whether various metabolites intermediate in major metabolic pathways when delivered to the lens in place of glucose could support cation transport across the limiting barriers in the lens.

Material and Methods

The methods employed have been described in greater detail elsewhere.[3] Fresh rabbit lenses were placed in three ml. of a solution containing the substance under study, refrigerated at O°C. for 41 to 44 hours, analyzed immediately, or incubated at 37°C. for an additional six hours. The basic solution was a modified Tyrode's solution containing added bicarbonate, glucose, and potassium.[3] Where substitutions for glucose were made 0.01 M. concentrations were utilized. Generally, the two lenses from the same animal were compared, one of the pair being analyzed after refrigeration, the other after refrigeration and subsequent incubation at 37°C. Results are generally expressed as percent recovery of the normal cation distributions.[3]

Where the penetration into the lens of isotopically marked metabolites was to be determined the isolated lenses were placed in three ml. of media containing known amounts of C^{14} labeled substrate, in tracer quantities, refrigerated at O°C. for 24 hours, and subsequently incubated at 37°C. for an additional six hours. At the end of this period the lenses were thoroughly rinsed with a medium of similar composition but devoid of isotope, weighed, frozen rapidly, and divided equatorially. The capsules were removed from the separate sections and transferred onto one inch planchets of aluminum construction for counting with an end-window counter. The entire nucleus plus cortex was macerated, spread evenly over a one inch planchet, and counted after drying for one hour at 105°C.

Standard curves were obtained by spreading decapsulated lenses on planchets in the aforementioned manner, adding known amounts of C^{14} labeled metabolite, thoroughly mixing, and counting as previously described. Standard curves for capsules were not obtained, but the assumption was made that the counting efficiency was in the same range as that of the macerated lens. Counting efficiency was determined by comparison with end-window counting of infinite thickness barium carbonate.*

* From the John E. Weeks Memorial Laboratory, Department of Ophthalmology, University of Oregon Medical School, Portland, Oregon. Supported by Grant B-187 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda 14, Maryland.

† Part of a thesis submitted by Dr. Heinrichs to the Graduate Council of the University of Oregon Medical School in partial fulfillment of the requirements for the degree of Master of Science.

* We wish to express our appreciation to Dr. John T. Van Bruggen and Miss Jean Scott for the valuable assistance given us during the course of this experiment.

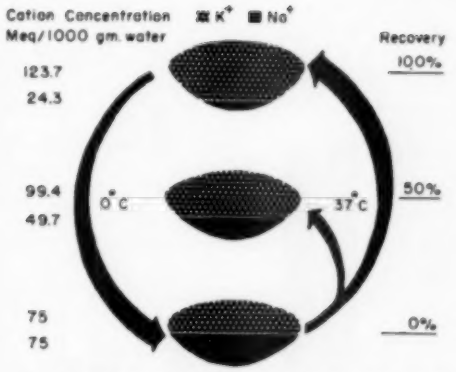

Fig. 1 (Heinrichs and Harris). Schematic representation of the changes in cation concentration of rabbit lenses during refrigeration at 0°C. and subsequent incubation at 37°C.

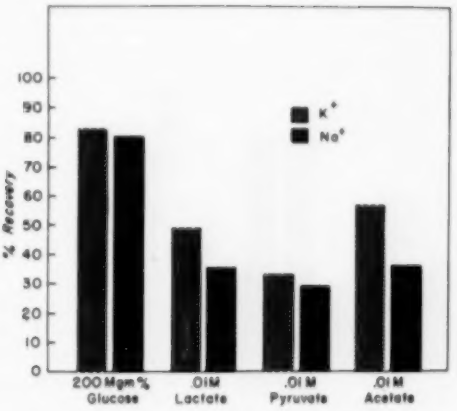

Fig. 2 (Heinrichs and Harris). Effect of replacement of glucose by lactate, pyruvate, or acetate on cation recovery of rabbit lenses during incubation at 37°C. following a cold-induced cation shift (each bar represents the average of at least 10 experiments involving 10 pairs of lenses).

Results

The effect on cation recovery of substituting for glucose various metabolites which may be metabolized via the citric-acid cycle

Glucose was replaced by various metabolites which may be metabolized via the citric-acid cycle. Pyruvate, acetate, lactate, alpha-keto-glutarate, citrate, and oxaloacetate were added as a possible energy source.

With none of the above compounds was cation recovery observed to approach that obtained when glucose was added (figs. 2 and 3). Citrate appeared to exert a deleterious effect, cation recovery being virtually abolished, and a marked hydration of the lens observed (fig. 4). Citrate is known to bind calcium effectively and the observed effect is probably similar to that seen when calcium is deleted from the incubating medium.[4]

The effect on cation recovery of substituting for glucose metabolites which may be metabolized via the hexose-monophosphate shunt

Two compounds, d-ribose and gluconate, were substituted for glucose in the modified Tyrode's solution and cation recovery measured in the usual manner. Lenses of such a size as to require added glucose were utilized throughout. Neither ribose nor gluconate supported cation recovery so effectively as did glucose (fig. 5).

Incorporation of labeled substrates into the lens during incubation at 37°C.

The aforementioned results indicated quite clearly that the major metabolites of glucose

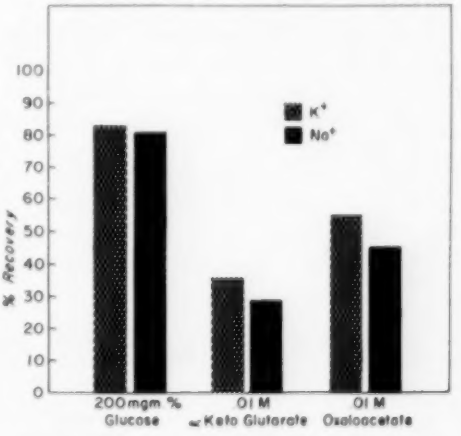

Fig. 3 (Heinrichs and Harris). Effect of replacement of glucose by alpha-keto-glutarate or oxaloacetate on cation recovery of rabbit lenses during incubation at 37°C. Following a cold-induced cation shift (each bar represents the average of at least 10 experiments involving 10 pairs of lenses).

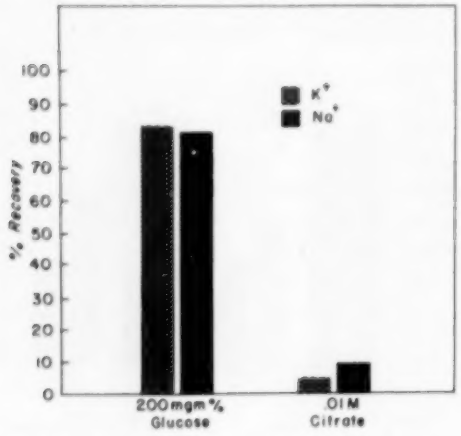

Fig. 4 (Heinrichs and Harris). Effect of replacement of glucose by citrate on cation recovery of rabbit lenses during incubation at 37°C. Following a cold-induced cation shift (each bar represents the average of at least 10 experiments involving 10 pairs of lenses).

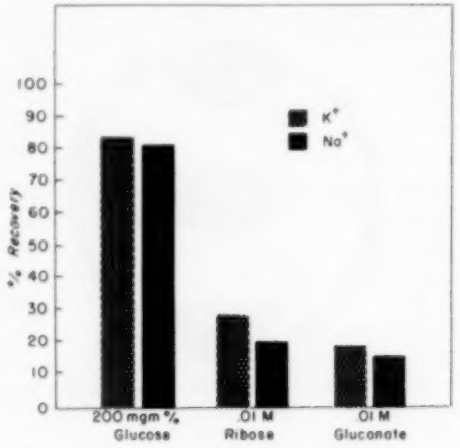

Fig. 5 (Heinrichs and Harris). Effect of replacement of glucose by ribose or gluconate on cation recovery of rabbit lenses during incubation at 37°C. following a cold-induced cation shift (each bar represents the average of at least 10 experiments involving 10 pairs of lenses).

when delivered to the lens were incapable of supporting lens functions as have been measured. Since it is known that glucose is preferentially moved across the lens surface it became imperative to determine whether the various metabolites moved into the lens with the same facility as did glucose. To test this hypothesis the movement of isotopically marked glucose into the lens was compared with that of the smallest metabolite tested, the two carbon residue, acetate, also isotopically marked.

Approximately 4.5 to 6.0 percent of the activity of the added acetate was recovered in the lens structures (table 1). The majority of the activity was found in the lens substance, but significant activity was recovered in the capsule. The anterior capsule in each instance was more active by a factor of two to four than the posterior capsule. Per unit weight the activity of the capsule far exceeded that of the lens substance.

On the other hand 8.5 to 9.5 percent of the activity of the added labelled glucose was incorporated into the lens at the end of the six hours of incubation at 37°C. following refrigeration at 0°C. for 24 hours (table 2). Again the activity of the anterior capsule exceeded that of the posterior capsule and, per unit weight, this activity of the capsule was far greater than that of the lens substance.

TABLE 1

INCORPORATION OF ACTIVITY INTO THE LENS DURING INCUBATION AT 37°C. FOLLOWING REFRIGERATION IN A MEDIUM CONTAINING C^{14} LABELLED ACETATE

Experiment	μC Labelled Acetate Added	Lens Substance	μC Recovered Anterior Capsule	Posterior Capsule	Total Percent Incorporation
1	0.2	0.0076	0.0040	0.0008	6.2
2	1.0	0.0302	0.0090	0.0040	4.3
3	1.5	0.0564	0.0121	0.0024	4.7
4	2.0	0.0833	0.0258	0.0068	5.8

TABLE 2

INCORPORATION OF ACTIVITY INTO THE LENS DURING INCUBATION AT 37°C. FOLLOWING REFRIGERATION IN A MEDIUM CONTAINING GLUCOSE UNIFORMLY LABELLED WITH C^{14}

Experiment	μC Labelled Glucose Added	Lens Substance	μC Recovered		Total Percent Incorporation
			Anterior Capsule	Posterior Capsule	
1	0.4	0.0295	0.0051	0.0034	9.5
2	1.0	0.0696	0.0115	0.0050	8.6

Discussion

It is obvious that the ability of any nutrient to serve as a source of energy will require that:

1. The metabolic pathways necessary for its utilization be present.

2. The substance be delivered to the site of utilization in sufficient quantity.

The pathways in the lens by which glucose is metabolized and in which oxygen participates are still not known with certainty. The glucose consumption of the lens is known to be small.[5] Its oxygen consumption is so low that measurement by the usual Warburg techniques is extremely difficult and subjected to high error. Indeed, some[6] have suggested that oxygen consumption is nonenzymatic but this does not appear to be true.[7] Present knowledge of these pathways in the lens is best summarized as follows:

First, the studies of Green and his associates have demonstrated that the classical anaerobic pathway, the Embden-Meyerhof cycle, appears to be followed in lens homogenates.[7-9]

Second, there is evidence that the cytochrome system of enzymes is present in the lens although in lesser concentration than in other tissues.[10]

Third, the participation of the citric-acid cycle in the lens is not certain. Bovine lens homogenates have been found to oxidize certain intermediates of the citric-acid cycle.[11] However, the intact lens does not appear to oxidize isotopically labelled pyruvate to carbon dioxide to any appreciable extent.[12]

Fourth, the hexose-monophosphate shunt may be followed in the lens. Thus, certain supposed intermediates of this pathway, that is, glucose-6-phosphate and 6-phosphogluconate, are utilized by lens homogenates.[13]

Most of these data have of necessity been obtained from studies of lens homogenates. To what extent it can be quantitatively applied to the intact structure is not certain. Indeed measurable changes in physiologic function as measured by cation transport attend even slight mechanical disturbance of the lens.[13] Changes in metabolic pathways must be considered when structural integrity is disrupted. For example, when the lens capsule is nicked, the rate of oxygen consumption immediately rises.[5] For this reason any postulated mechanism must ultimately be proved to occur in the intact structure.

The observations herein reported indicate that metabolites delivered to the lens do not support lens function as herein measured possibly because they do not penetrate to the site of utilization in sufficient quantity. The finding that glucose enters the lens more readily than acetate (on the basis of molecular size one would anticipate the opposite) supports the hypothesis that glucose enters the lens by metabolic mediation.[14] The failure of the various metabolites to support cation transport as well as glucose must, in the light of these experiments, be considered to be due largely to insufficient penetration of these metabolites into the lens. No definite conclusion concerning the specific pathways of glucose degradation can be drawn from these studies.

Summary

1. Compounds which may be metabolized

via the citric-acid cycle did not support cation recovery following the cold-induced cation shift in rabbit lenses as well as does glucose.

2. Compounds which may be metabolized via the hexose-monophosphate shunt did not support cation recovery following the cold-induced cation shift in rabbit lenses as well as does glucose.

3. Glucose was incorporated into the intact rabbit lens in vitro to a greater extent during incubation at 37°C. following refrigeration than was acetate.

4. The significance of these findings is discussed.

3181 S.W. Sam Jackson Park Road (1)

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13. Harris, J. E., and Nordquist, L. T.: Factors effecting the cation and water balance of the lens. Tr. XVII Internat. Cong. Ophth., 1954, pp. 1002-1012.

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Discussion

Dr. Daniel J. Heinrichs: The phosphorylated compounds intermediate in glucose metabolism have not been employed in our present studies. Previously we have substituted for glucose, glucose-6-phosphate, and have observed that this compound does not support cation transport in the intact rabbit lens in vitro to the same extent as does glucose. However, additional studies utilizing various phosphorylated intermediates should indeed yield valuable information germane to the present studies.

We have restricted the present studies to the use of an artificial medium, a modified Tyrode's solution. Certainly aqueous humor as an incubating medium would provide an environment more closely resembling in vivo conditions. Perhaps in the future such studies utilizing aqueous humor as an incubating medium may be possible.

We are anticipating additional studies involving the penetration of various metabolites into the lens. Studies are underway of the effect of insulin on the penetration of glucose across the lenticular barriers in vitro both in normal and alloxan diabetic rabbits.

I would like to express my appreciation to Dr. Wachtl for his discussion of the paper and his helpful suggestions.

TWENTY-FIFTH MEETING

of the

Association for Research in Ophthalmology, Inc.

Proceedings

Business Session

Auditor's Report

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Geographical List

Provisional Members

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FREDERICK C. CORDES

DERRICK T. VAIL

Chicago, Illinois

June 11, 12, 13, and 14, 1956

BUSINESS SESSION

CHAIRMAN CLARK: The business session will please come to order.

The minutes of the last business meeting of the Association for Research in Ophthalmology were published in the Supplement of THE AMERICAN JOURNAL OF OPHTHALMOLOGY and have been distributed. Do I hear a motion that they be accepted as published?

VOICE: I so move.

(The motion was duly seconded, was put to a vote, and was carried unanimously.)

CHAIRMAN CLARK: The next matter is the report of the secretary, Dr. Johnson.

SECRETARY JOHNSON: As of December 31, 1955, there were 822 paid members. At the Trustees' meeting Monday morning 116 new members were admitted to membership. This makes a total of 938 members.

CHAIRMAN CLARK: I think that speaks well for our very efficient secretary.

Next will be the treasurer's report.

SECRETARY JOHNSON: As of December 31, 1954, cash in hand in the treasury was $2,590. As of December 31, 1955, there was $2,045.97 cash on hand in the treasury.

DR. BURIAN: I move that the report be accepted.

(The motion was duly seconded, was put to a vote, and was carried unanimously.)

CHAIRMAN CLARK: The auditing committee has sent in a written report. They have examined the books, and find them to be in order. Dr. Brittain Payne is chairman.

Is Dr. Payne in the room? Not being here, do I hear a motion that we accept his report as recorded?

DR. BURIAN: I so move.

(The motion was duly seconded, was put to a vote, and was carried unanimously.)

CHAIRMAN CLARK: Next is the report of the nominating committee. The secretary will read the report.

SECRETARY JOHNSON: The nominating committee recommended as a candidate for the new trustee the name of Harold Falls, and for secretary-treasurer, L. V. Johnson.

CHAIRMAN CLARK: Are there any nominations from the floor?

VOICE: I move that nominations be closed and that the officers be elected by acclamation.

(The motion was duly seconded, was put to a vote, and was carried unanimously.)

CHAIRMAN CLARK: The following members have been elected to honorary membership in this association: Dr. F. Phinizy Calhoun, Sr., Atlanta, Georgia. Dr. Arthur J. Bedell, Albany, New York. Dr. Frederick C. Cordes, San Francisco. Dr. Derrick Vail, Chicago. Dr. Robert Masters, Indianapolis. (Applause.)

As you all know, this society sustained a great loss in the passing of Dr. Jonas Friedenwald since our last meeting. The friends of Dr. Friedenwald have instituted action to establish a lectureship in Dr. Friedenwald's honor. This committee, with Dr. David Cogan as chairman, will make their report at this time.

DR. DAVID COGAN: This fund was set up, as you know, at the request of the officers of the association immediately after Dr. Friedenwald's death, and served as a repository for contributions from members of this association and others who want to establish some memorial to Jonas Friedenwald.

As a result, a temporary fund has been set up in a bank in Cambridge, Massachusetts, where it is drawing three percent interest. To that fund has been added now around $5,300. In addition, there is a firm promise of $2,000 from one other source. Thirdly, a large addition to the fund is possible in the near future. We are not at liberty to describe it but it will be a substantial addition to the fund.

The purposes of the fund have not been entirely established. The trustees, in their meeting, suggested a lectureship. I think the suggestions are still open, and if any of you wish to communicate with Dr. Kinsey or Dr. Becker or me, we will transmit your suggestions. By the time of our meeting next year presumably there will be a formulation of a definitive plan for the most fitting steps to the memory of Jonas Friedenwald.

The next meeting of the Association for Research in Ophthalmology will be held in New York City in conjunction with the American Medical Association, June 2-6, 1957.

Is there any other business to come before the association before we close our executive session? There being none, I declare it closed, and we will proceed with the scientific program.

AUDITOR'S REPORT

Association for Research in Ophthalmology, Inc.

April 16, 1956

To the Members of The Board of Trustees
Association for Research in Ophthalmology, Inc.
Cleveland, Ohio

Dear Sirs:

We have examined the cash basis accounting records of the Association for Research in Ophthalmology, Incorporated, as of December 31, 1955 and the related transaction of receipts and disbursements for the year then ended. The examination was made in accordance with generally accepted auditing standards and included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

As of January 1, 1955, Dr. Lorand V. Johnson of 10515 Carnegie Avenue, Cleveland, Ohio, became the secretary-treasurer and it was to his offices that all records of the Association were therefore subsequently transferred. Funds forwarded from New Orleans in two remittances of $1,000.00 and $514.20 which were deposited on January 25th and June 9th respectively in the Cleveland Trust Co., Cleveland, Ohio. The audit included a review of the financial transactions prior to and subsequent to the transfer of the association's headquarters to Cleveland.

Deposited to the General fund was the balance of $15.37 which had previously been retained separately in the Proctor Medal Fund. As directed at the meeting of the Board of Trustees in June of 1952 all interest income from the Proctor Medal Fund was to be deposited in the general fund and all disbursements authorized under the Proctor Medal Fund were similarly to be disbursed through the general fund. The $10,000.00 of 2% U. S. Treasury bonds were forwarded to Dr. Lorand V. Johnson for safe keeping in Cleveland under authorization letter dated March 16, 1955.

Individual membership cards were inspected and found to be satisfactorily maintained and the financial transactions were verified from the various pertinent supporting documents.

Effective for the year 1956 individual dues of educational members are to be increased to $3.00, that of active members to $10.00 and that of sustaining members are to remain at $25.00.

Despite the fact that no confirmation of dues in arrears was made directly with the members, in our opinion the accompanying statements present fairly the fund balances of the Association for Research in Ophthalmology, Incorporated as of December 31, 1955, and the receipts and disbursements for the year then ended, in conformity with generally accepted cash basis accounting principles and applied on a basis consistent with that of the prior year. Since the amount of dues in arrears does not appear large, the exception of not confirming such unpaid amounts with the members is not of sufficient importance to negate this opinion.

Very truly yours,

SANFORD R. FEIN.

Cash and Securities in Funds

	General Fund	Proctor Medal Fund		Total
Cash 12/31/54	$ 2,045.97	$ 15.37		$ 2,061.34
Cash 12/31/55	2,590.19	—0—	(1)	2,590.19
Increase or Decrease	$ 544.22	(15.37)		528.85
Securities	—	$10,184.63		$10,184.63
		$10,184.63	(1)	10,184.63
Total Cash & Securities as of 12/31/55			(1)	12,774.82

Statement of Cash Receipts and Disbursements

For Year Ended December 31, 1955

		General Fund
Cash Balance on January 1, 1955 (Deposited and Undeposited funds)		$ 2,045.97
Add: Receipt of Dues and Other Deposits		
Dues Collected (Page 5)	$ 5,885.00	
Interest from Government Bonds[1]	375.00	
Banquet Proceeds	588.50	
Supplements	12.00	
Deposit—Transferred from Proctor Medal Fund	15.37	$ 6,875.87
Total of Cash Funds Accounted For		$ 8,921.84
Less: Disbursements		
Convention Expense:		
Dinners	$596.00	
Programs, notices, tickets, etc.	415.97	
Expenses of secretary	250.00	$ 1,261.97
Publications[2]		2,000.00
Stationery, Supplies & Printing		865.95
Postage & Express		118.11
Insurance Bond—5,000.00 (Secretary-Treasurer)		12.50
Bank Charges		14.92
Dues to International Federation of Ophthalmology		319.25
Express and Crating Charges		44.76
Telephone		75.17
Secretarial Service[3]		900.00
Salary[4]	$ 50.00	
Less: Social Sec. & Withholding	10.00	40.00
Social Security & Withholding Paid		33.00
Sundry		17.86
Expenses—Board of Trustees in Chicago		43.71
Contributions		375.00
Audit Charges		193.00
Refunds for Banquet		16.50 $ 6,331.70
Cash Balance and In Bank on December 31, 1954		$ 2,590.14

[1] Includes deposit of $125.00 for balance of 1954 coupons on U. S. Treasury Bonds paying 2½% due 1967-72 having a carrying cost of $10,184.63.

[2] Payments for 1954-55 publications of the American Journal of Ophthalmology.

[3] Paid through December 31, 1955.

[4] Represented December 1954 salary paid in January 1955.

Statement of Dues Collected
For Year Ended 12/31/55

Dues collected for prior years		
Year 1948— 1 active member @ $5.00	$ 5.00	
Year 1949— 12 active members @ $5.00	60.00	
Year 1950— 15 active members @ $5.00	75.00	
Year 1951— 17 active members @ $5.00	85.00	
Year 1951— 2 educational members @ $2.00	4.00	
Year 1952— 22 active members @ $5.00	110.00	
Year 1952— 7 educational members @ $2.00	14.00	
Year 1953— 40 active members @ $5.00	200.00	
Year 1953— 6 educational members @ $2.00	12.00	
Year 1954—105 active members @ $5.00	525.00	
Year 1954— 8 educational members @ $2.00	16.00	$ 1,106.00
Dues collected for current year		
633 active members @ $5.00	$ 3,165.00	
3 active members @ $10.00	30.00	
48 educational members @ $2.00	96.00	
2 educational members @ $3.00	6.00	
33 sustaining members @ $25.00	825.00	$ 4,122.00
Dues collected for subsequent years		
7 active members @ $5.00	$ 35.00	
6 active members @ $10.00	60.00	
1 education member @ $2.00	2.00	
2 educational members @ $3.00	6.00	
2 sustaining members @ $25.00	50.00	$ 153.00
Dues collected from provisional members		
42 active members @ $10.00	$ 420.00	
3 educational members @ $3.00	9.00	
3 sustaining members @ $25.00	75.00	$ 504.00
Total dues collected in year 1955		$ 5,885.00

Membership summary by years through December 31, 1955

Years Ended December 31	Total Members	Years Ended December 31	Total Members
1955	[1]822	1942	281
1954	[2]815	1941	279
1953	[2]713	1940	270
1952	[2]672	1939	268
1951	[2]556	1938	272
1950	[2]509	1937	249
1949	474	1936	240
1948	422	1935	245
1947	306	1934	230
1946	324	1933	219
1945	[4]—	1932	203
1944	283	1931	193
1943	[4]—	1930	134

[1] Includes 774 active, sustaining and educational members and 48 provisional members.
[2] Official membership whether dues were paid or not.
[3] Includes only paid up members.
[4] Statistical information not available.

Including dues of 2 active members and 1 educational member received in 1954, the paid official membership including all categories was 722 for the calendar year 1955.

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1956

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Ludwig von Sallmann
V. Everett Kinsey
Kenneth C. Swan
David G. Cogan
George Wald
W. Morton Grant

MEMBERS

A

Abraham, Samuel V., 6363 Wilshire Blvd., Los Angeles 36, Calif.
Abrahamson, Ira A., 925 Union Trust Bldg., Cincinnati, Ohio.
Abrams, Henry, 195 Nassau St., Princeton, N.J.
Addington, Charles H., 675 Delaware Ave., Buffalo, N.Y.
Adler, Francis H., 313 S. 17th St., Philadelphia, Pa.
Aiken, Samuel D., 1154 Montgomery Dr., Santa Rosa, Calif.
Alberstadt, Norbert F., 1106 G. Daniel Baldwin Bldg., Erie, Pa.
Alexander, Rose C., Univ. Hospital, Dept. Ophth., Iowa City, Iowa.
Alfano, Joseph E., 122 S. Michigan Ave., Chicago, Ill.
Allen, Henry Freeman, 200 Beacon St., Boston 16, Mass.
Allen, James H., 1430 Tulane Ave., New Orleans, La.
Allen, Lee, Univ. Hosp., Iowa City, Iowa.
Allen, Merrill J., Div. Optometry, Ind. Univ., Bloomington, Ind.
Allen, Robert T., Recent address unknown.
Alliband, George T., 1020 Med. Arts Bldg., Omaha, Neb.
Alvaro, Moacyr E., 1151 Rua Consolacoa, Sao Paulo, Brazil.
Alvis, Edmund B., 100 North Euclid, St. Louis 8, Mo.
Anderson, Banks, Duke Hosp., Durham, N.C.
Anderson, Thomas W., 1110 Harvard Ave., Seattle 22, Wash.
Armstrong, Richard C., 595 E. Colorado St., Pasadena 1, Calif.
Apter, Julia T., 1902 Indianapolis Blvd., Whiting, Ind.
Arnott, George P., Dept. Ophth., Univ. Hosp., Iowa City, Iowa.
Arribas, Neva P., Dept. of Ophth., Ind. Univ. Med. Center, Indianapolis, Ind.
Ascher, Charles K. W., 1404 East McMillan, Cincinnati, Ohio.
Askovitz, Samuel I., Ophth. Research Lab., Albert Einstein Med. Center, Northern Div., York and Tabor Rds., Philadelphia 41, Pa.
Atkinson, George S., 418 High Ave., Oskaloosa, Iowa.
Atkinson, Walter S., 129 Clinton St., Watertown, N.Y.
Auten, Hanford L., Hitchcock Clinic, Hanover, N.H.

B

Bahn, Charles A., 1210 Maison Blanche Bldg., New Orleans, La.
Bahn, Gustav C., 1210 Maison Blanche Bldg., New Orleans, La.
Bair, Hugo L., Mayo Clinic, Rochester, Minn.
Baird, J. Mason, 235 Strickler Bldg., 1293 Peachtree St., N.E., Atlanta 5, Ga.
Baisinger, W. F., 2010 17th St., Bakersfield, Calif.
Balazs, Endre A., 30 Chambers St., Boston, Mass.
Balding, Laurence G., 101 S. Madison, Pasadena 1, Calif.
Ball, Donald N., 421 Wainwright Bldg., Norfolk 10, Va.
Ball, William L., 514 W. 3rd Ave., Warren, Pa.
Ballintine, Elmer J., 10515 Carnegie Ave., Rm. 224, Cleveland 6, Ohio.
Barber, Acleta N., Dept. of Pathology, LSU Med. School, New Orleans, La.
Barkan, Hans, 2400 Clay St., San Francisco 15, Calif.
Barkan, Otto, 490 Post St., San Francisco 2, Calif.
Barrere, Luciano E., Acisclo Villaran 356, Lima, Peru, S.A.
Barnett, Irving F., 30 N. Michigan Ave., Chicago 2, Ill.
Bartlett, Robert E., Wadsworth V.A. Hosp., Wilshire & Sawtelle, Los Angeles, Calif.
Bassen, Edward J., 70 E. 66th St., New York, N.Y.
Battles, Morris L., 370 E. Town St., Columbus, Ohio.
Beaman, Thomas C., 1569 Wentworth, Calumet City, Ill.
Becker, Bernard, 640 S. Kingshighway Blvd., St. Louis 10, Mo.
Bedell, Arthur J., 344 State St., Albany, N.Y.
Beetham, W. P., 108 Bay State Rd., Boston, Mass.
Bell, Dudley P., 411 30th St., Oakland, Calif.
Bell, Richard P., Jr., 11621 Detroit Ave., Cleveland 2, Ohio.
Bellows, John G., 30 N. Michigan Blvd., Chicago 2, Ill.
Benedict, Walter H., Shelbourne Towers, Knoxville, Tenn.
Benedict, Walter H., Jr., Shelbourne Towers, Knoxville, Tenn.
Benedict, W. L., Mayo Clinic, Rochester, Minn.
Bennett, Arthur L., 147 Linwood Ave., Buffalo, N.Y.
Benson, Clifton E., 245 4th St. Bldg., Bremerton, Wash.
Benton, Curtis D., Jr., 15 S.E. 16th St., Ft. Lauderdale, Fla.
Berens, Conrad, 708 Park Ave., New York, N.Y.
Bergmann, Robert B., 1225 E. Montauk Hwy., West Islip, N.Y.
Berry, A. Erwin, 1846 Teaneck Rd., Teaneck, N.J.
Bettman, Jerome W., 2400 Clay St., San Francisco 15, Calif.
Bierman, Edward O., 5885 San Vicente, Los Angeles, Calif.
Billings, Robert J., Jenkins Arcade Bldg., Pittsburgh 22, Pa.
Bizzell, James W., 314 Borden Bldg., Goldsboro, N.C.
Blake, Eugene M., 303 Whitney Ave., New Haven, Conn.
Blankstein, Samuel S., 208 E. Wisconsin Ave., Milwaukee, Wis.
Blazar, Howard A., 520 Beacon St., Boston 15, Mass.
Blodi, Frederick C., Univ. Hosp., Iowa City, Iowa.
Bloomberg, Louis, 508 Home Savings & Loan Bldg., Youngstown 3, Ohio.
Bloomenthal, Sanford R., Recent address unknown.
Bloomveld, Sylvan, 1010 5th Ave., New York 28, N.Y.
Blum, John, 44, Boulevard des Tranchees, Geneva, Switzerland.
Boland, W. T., 378 W. Church St., Elmira, N.Y.
Boles, W. M., 530 St. Peter St., New Orleans 16, La.
Bonaccolto, Girolamo, 123 E. 61st St., New York 21, N.Y.
Bonadia, Calogero, 1822 70th St., Brooklyn 4, N.Y.
Borley, W. E., 655 Sutter, San Francisco 2, Calif.
Boruchoff, Saul A., 118 E. 19th St., New York 3, N.Y.
Bounds, George W., Jr., 136 Windsor Dr., Nashville, Tenn.
Boyd, James L., 100 W. 59th St., New York 19, N.Y.
Boyer, Harvey K., Dept. Ophth., Calif. Univ. Med. Center, San Francisco, Calif.
Boyes, Truman L., 654 Madison Ave., New York, N.Y.
Braley, Alson E., Univ. Hosp., Iowa City, Iowa.
Brandenburg, K. C., 110 Pine Ave., Long Beach 2, Calif.
Brandwan, Samuel R., 804 Rose Bldg., Cleveland, Ohio.
Brault, Jules, 418 E. Sherbrooke St., Montreal, Canada.
Braw, Solomon S., 5575 N. Park Ave., Philadelphia, Pa.
Breakey, Arnold S., 708 Park Ave., New York 21, N.Y.
Brecher, Gerhard A., Ohio State Univ., Columbus, Ohio.
Breinin, Goodwin M., 3 E. 66th St., New York 21, N.Y.
Brennan, James W., 88 W. Utica St., Buffalo, N.Y.
Bribach, E. J., 603 Commercial St., Atchison, Kan.
Bristow, James, 1718 Hampton St., Columbia, S.C.
Bronk, Henry N., 708 Park Ave., New York 21, N.Y.
Brown, Albert L., 1137-38 Carew Tower, Cincinnati, Ohio.
Brown, David V. L., Sch. of Aviation Medicine, Randolph Field, Tex.
Browning, Carroll W., 101 N. Zangs Blvd., Dallas, Tex.
Browning, Charles W., 919 Taylor St. Bldg., Portland, Ore.
Bruner, Abram B., 1214 Guardian Bldg., Cleveland, Ohio.
Bryan, James H., 3720 Washington Blvd., St. Louis, Mo.
Buckhaults, W. W., 103 E. Jones St., Savannah, Ga.
Buckheit, Rudolph G., 1920 Seneca St., Buffalo 20, N.Y.
Buckley, Eugene T., 131 Fulton Ave., Hempstead, L.I., N.Y.
Budd, Francis X., United Office Bldg., Cleveland, Ohio.
Buesseler, John A., 110 E. Main St., Madison 3, Wis.
Burch, Edward P., 424 Hamm Bldg., St. Paul, Minn.
Burdin, John J., 105 Seville Blvd., Lafayette, La.
Burian, Hermann M., Dept. of Ophth., Univ. Hosp., Iowa City, Iowa.
Burke, John W., 2000 Massachusetts Ave., N.W., Washington 6, D.C.
Burman, Daniel, 2001 Grand Concourse, Bronx, N.Y.
Burnett, Arthur B., 310 Burr Bldg., New Castle, Ind.
Burr, Sherwood P., Jr., 43 E. Jackson St., Tucson, Ariz.
Bussey, Frank R., 375 E. Main, Bay Shore, N.Y.
Butler, J. B. V., 919 Taylor St. Bldg., Portland, Ore.
Buxeda, Roberto, 303 De Diego Ave., Santurce, Puerto Rico
Byers, Jerome Leon, 1430 Tulane Ave., Dept. of Ophth., Tulane Medical School, New Orleans, La.
Byrnes, Victor A., Dept. of the Air Force, Headq. USAF, Washington 25, D.C.

C

Caccamise, W. C., 314 Medical Arts Bldg., 277 Alexander St., Rochester 7, N.Y.
Calhoun, F. Phinizy, 478 Peachtree St., Atlanta 3, Ga.
Calhoun, F. Phinizy, Jr., 478 Peachtree St., Atlanta, Ga.
Calkins, Larry L., 411 Nichols Rd., Kansas City 12, Mo.
Callahan, Alston, 903 S. 21st St., Birmingham, Ala.
Campion, George S., 490 Post St., San Francisco 2, Calif.
Carmichael, Paul L., 1930 Chestnut St., Philadelphia, Pa.
Carrasquillo, Honorio F., Med. Arts Bldg., Puerto de Tierra, Puerto Rico.
Carroll, Frank D., 635 W. 165th St., New York 32, N.Y.
Carter, George Z., 708 Park Ave., New York 21, N.Y.
Cassady, J. Vernal, 921 Lincoln Way E., South Bend, Ind.
Casten, Virgil G., 412 Beacon St., Boston 15, Mass.
Castroviejo, Ramon, 9 E. 91st St., New York 28, N.Y.
Caylor, Robert N., Med. Arts Bldg., El Paso, Tex.
Chalfant, W. Paxson, Jr., 1228 16th St., N.W., Washington 6, D.C.
Chamberlain, Webb P., Jr., 1324 Hanna Bldg., Cleveland 2, Ohio.
Chambers, Arthur L., II, 27 Ludlow St., Yonkers, N.Y.
Chandler, Paul A., 5 Bay State Rd., Boston, Mass.
Chan-Pong, Norman R., 88 Frederick St., Port of Spain, Trinidad, B.W.I.
Chickering, Donald H., 262 E. Market St., Warren, Ohio.
Chisholm, Julian F., Jr., 34½ Beacon St., Boston, Mass.
Cholst, Mortimer, 1 Nevins St., Brooklyn, N.Y.
Christensen, Leonard, Univ. Oregon Med. Sch. Hosp. and Clinics, Portland, Ore.
Christiansen, Gordon S., Recent address unknown.
Cibis, Paul A., Dept. of Ophth., Sch. Med., Washington Univ., St. Louis, Mo.
Cinotti, Alfonse A., 3285 Hudson Blvd., Jersey City, N.J.
Clark, Graham, 635 W. 165th St., New York 32, N.Y.
Clark, W. B., 211 S. Saratoga, New Orleans, La.
Clothier, W. L., 407-411 Kane Bldg., Pocatello, Idaho.
Cloutier, Roland, 1538 Oest Rue, Sherbrooeke, Canada.
Cogan, David G., 243 Charles St., Boston, Mass.
Cole, Helen G., 780 Park Ave., New York, N.Y.
Cole, John G., 780 Park Ave., New York, N.Y.
Coles, Robert S., 1148 5th Ave., New York, N.Y.
Conner, W. C., P.O. Box 1959, 1400 Henderson, Fort Worth, Tex.
Constantine, F. H., 30 W. 59th St., New York 19, N.Y.
Constantine, K. W., 229 Barton Ave., Palm Beach, Fla.
Cordes, Frederick C., 384 Post St., San Francisco 8, Calif.
Cosgrove, Kingsley W., 111 E. Capitol Ave., Little Rock, Ark.
Courtney, R. H., 501 Franklin St., Richmond, Va.
Courvoisier, Earl A., Recent address unknown.
Covey, John K., 140 W. High St., Bellefonte, Pa.
Cowan, Alfred, 1930 Chestnut St., Philadelphia, Pa.
Cowen, Jack P., 109 N. Wabash Ave., Chicago 2, Ill.
Craig, Paul C., 232 N. 5th St., Reading, Pa.
Crawford, H. C., 705 Doctors Bldg., 478 Peachtree St., Atlanta 3, Ga.
Crawford, Joseph Wm., 490 Post St., San Francisco 2, Calif.
Crawford, Walter J., 4296 Orange St., Riverside, Calif.
Cregar, John S., 440 Harrison St., East Orange, N.J.
Cuadra, Marie K., Apartado No. 521, Managua, Nicaragua
Culler, Arthur M., 150 E. Broad St., Columbus, Ohio.
Cumming, Edith L. Wentworth, Valley Cottage, N.Y.
Cunningham, John C., 149 Swinooski Ave., Burlington, Vt.
Curtin, Brian J. H., 1777 Grand Concourse, New York 53, N.Y.
Cushman, Beulah, 25 E. Washington St., Chicago 2, Ill.
Culter, Morton, 1515 Addison Ave., E. Twin Falls, Idaho.

D

Daily, Louis, Jr., 1117 Med. Arts Bldg., Houston, Tex.
D'Amico, Thomas V., 85 Passaic Ave., Passaic, N.J.
Dasilva, Antonio I., Rua do Ouro 167 Belo Horizonte, Minas Gerais, Brazil, S.A.
Davidson, Bernard, 14435 Hamlin St., Van Nuys, Calif.
Davies, Robert H., 634 Washington Rd., Pittsburgh 28, Pa.
Day, R. M., 635 W. 165th St., New York 32, N.Y.
Dayton, Glenn O., Jr., 1052 W. 6th, Los Angeles, Calif.
Dean, Alfred, Sagola, Mich.
de Andrade, Cesario, Bahia, Brazil.
De Francois, Walter, 122 S. Michigan Ave., Chicago 3, Ill.
Dellaporta, Angelos, 3435 Main St., Buffalo 14, N.Y.
DeLong, Samuel L., N.E. Corner 20th & Chestnut Sts., Philadelphia 3, Pa.
Demorest, Byron H., 640 S. Kingshighway, St. Louis 10, Mo.
Dennis, Richard H., 33 College Ave., Waterville, Me.
De Ocampo, Geminiano, 654 Isaac Peral, Manila, P.I.
de Susto-Nagy, Ilona K., 158 Whitney Ave., New Haven 11, Conn.
De Voe, Arthur G., 30 W. 59th St., New York 32, N.Y.
De Witt, Edward N., 881 Lafayette, St., Bridgeport 4, Conn.
Dickson, Owen C., 2320 Channing Way, Berkeley, Calif.
Dillahunt, Jack A., 106 Girard Blvd., S.E., Albuquerque, N.M.
Dische, Zacharias, 630 W. 168th St., New York 32, N.Y.
Diskan, Samuel M., 1616 Pacific Ave., Atlantic City, N.J.
Dixon, Joseph M., 2156 Highland Ave. S., Birmingham, Ala.
Doherty, W. B., 150 W. 55th St., New York, N.Y.
Dolphin, Joseph M., Recent address unknown.
Downing, Arthur H., 406 6th Ave., Des Moines, Iowa.
Duane, Thomas D., Bedminster, Pa.
Duggan, John W., 10023 103 St., Edmonton, Alberta, Canada.
Duke-Elder, Lady Phyllis, London, England.
Duke-Elder, Sir Stewart, London, England.
Dunnington, John H., 635 W. 165th St., New York 32, N.Y.
Dunphy, Edwin B., 243 Charles St., Boston, Mass.
Durham, Davis G., Medical Arts Bldg., Wilmington, Del.
Dwiggins, Horace G., Veterans Hosp., Tuskegee, Ala.
Dyar, Edwin W., 3202 N. Meridian St., Indianapolis 8, Ind.
Dyson, Charles, 283 Queens Ave., London, Ontario, Canada.

E

Eager, R. F., 1862 Ferry St., Niagara Falls, Ontario.
Ehrenfeld, Edward, 185 Lexington Ave., Passaic, N.J.
Ellenberger, Carl, 14805 Detroit Ave., Lakewood 7, Ohio.
Ellerbrock, Vincent J., Sch. Optometry, Ohio State Univ., Columbus, Ohio.
Elliot, Alfred J., 170 St. George St., Toronto 5, Canada.
Ellis, Orwyn H., 523 W. 6th St., Los Angeles 14, Calif.
Ellis, Richard A., Spruce St. Med. Bldg., 269 S. 19 St., Philadelphia 3, Pa.
Elvin, Norman L., 309 Med. Arts Bldg., Winnipeg, Manitoba, Canada.
Emerson, George O., Jr., Recent address unknown.
Engel, Samuel, 350 Post St., San Francisco 8, Calif.
Epstein, Sidney S., 2021 Grand Concourse, New York, N.Y.
Esposito, Albert C. M., 1212 1st Huntington Natl. Bank Bldg., Huntington, W.Va.
Esterman, Benjamin, 54 E. 72nd St., New York 21, N.Y.
Eubank, W. R., 1404 Bryant Bldg., Kansas City, Mo.
Evans, Thomas M., Suite 100, 550 Grant St., Pittsburgh 19, Pa.
Evans, W. H., 510 Dollar Bank Bldg., Youngstown, Ohio.

F

Fair, John R., Meridian, Ga.
Falls, Harold F., Uni. Hosp., Ann Arbor, Mich.
Feinstein, Robert R., 37 W. 57th St., New York 19, N.Y.
Feldstein, Morris, 1225 Park Ave., New York 28, N.Y.
Ferrer, Horatio, Havana, Cuba
Fial, Edward A., 90 Berwyn Ave., Buffalo 15, N.Y.
Filkins, John C., 419 City Natl. Bank Bldg., Omaha, Neb.
Fine, Max, 655 Sutter St., San Francisco 2, Calif.
Fink, Austin, I., 110 Remsen St., Brooklyn 2, N.Y.
Fink, Walter H., 1921 Medical Arts Bldg., Minneapolis 2, Minn.
Finley, John K., 51 Kings Highway, W., Haddonfield, N.J.
Fisher, James A., Jr., Medical Arts Bldg., 601 Grand Ave., Asbury Park, N.J.
Fitzgerald, James R., 3215 W. North Ave., Chicago, Ill.
Fixott, R. S., 5700th Med. Sqn., Albrook, AFB, Canal Zone.
Flanagan, Roger M., Recent address unknown.

Fleming, David G., Dept. of Physiology, Univ. of Kansas, Lawrence, Kan.
Flom, Merton C., Univ. Calif., Sch. Optometry, Berkeley 4, Calif.
Flynn, Gregory E., 679 Juniper St., N.E., Atlanta 8, Ga.
Flynn, John E., Recent address unknown.
Foerster, Helenor C., 2529 Vallejo St., San Francisco 23, Calif.
Fonda, G. E., 84 Baltusrol Way, Short Hills, N.J.
Foote, Franklin M., 1790 Broadway, New York, N.Y.
Forrest, Robert L., U.S. Army, Camp Carson, Colo.
Fowler, James G., 412 Linwood Ave., Buffalo 9, N.Y.
Fralick, F. Bruce, 408 1st Natl. Bank Bldg., Ann Arbor, Mich.
François, Jules, 15, Place de Smet de Naeyer, Ghent, Belgium.
Franks, Myron B., 401-402 Hotel Jamestown Bldg., Jamestown, N.Y.
Freeman, Sheldon B., 196 Linwood Ave., Buffalo, N.Y.
Friedman, Benjamin, 8 E. 96th St., New York, N.Y.
Frink, Berton F., Suite 2, M & J Nelson Bldg., McAllen, Tex.
Frisch, Michael R., V.A. Center (Sawtelle) Los Angeles 25, Calif.
Fritschi, Ulrich A., 523 Medico Dental Bldg., Sacramento 14, Calif.
Frohring, W. O., Munn Rd., Newbury, Ohio.
Fry, Glenn A., Sch. Optometry, Ohio State Univ., Columbus, Ohio.

G

Gallardo, Edward, 153 Marquette St., La Salle, Ill.
Gans, Jerome A., 1020 Huron Rd., Cleveland 15, Ohio.
Gans, Morris E., 1621 Euclid Ave., Cleveland 15, Ohio.
Garron, Levon K., 426 17th St., Oakland 12, Calif.
Gehrsitz, Leta B., Recent address unknown.
Gettes, Bernard C., 1930 Chestnut St., Philadelphia 3, Pa.
Gifford, Harold, 1620 Med. Arts Bldg., Omaha, Neb.
Gilger, Anita P., 65 Skyview Dr., Seven Hills 31, Ohio.
Gill, King, Suite 14, Medical Center, 2414 Morgan Ave., Corpus Christi, Tex.
Gillette, David F., 109 S. Warren St., Syracuse, N.Y.
Gingrich, Wendell D., Univ. Texas Med. Sch., Galveston, Tex.
Ginsberg, Joseph, Recent address unknown.
Gipner, John F., 277 Alexander St., Rochester 7, N.Y.
Givner, Isadore, 108 E. 66th St., New York 16, N.Y.
Goar, Everett L., 1304 Walker Ave., Houston 2, Tex.
Goetzman, Arthur C., 1010 United Office Bldg., Niagara Falls, N.Y.
Gor, John L., 320 Wilshire Blvd., Santa Monica, Calif.
Goldberg, Bernard, 118 4th St., Kew Gardens 15, N.Y.
Goldberg, Sol, 8082 Jenkins Arcade, Pittsburgh, Pa.
Golden, Alfred, Dept. of Pathology, Wayne Univ., Col. of Med., Detroit, Mich.
Goodfellow, Thomas J., 20 Arcade Bldg., Saratoga Springs, N.Y.
Gordon, Dan M., 133 E. 58th St., Rm. 701, New York, N.Y.
Graham, John H., Parma Med. Center, 5500 Ridge Rd., Parma 29, Ohio.
Grana, Philip C., 401 Jenkins Bldg., Pittsburgh 22, Pa.
Grant, Hendrie W., 330 Lowry Med. Arts Bldg., St. Paul, Minn.
Grant, W. Morton, 243 Charles St., Boston 14, Mass.
Green, Harry, Wills Eye Hosp., Philadelphia 30, Pa.
Gricey, Edward W., 1022 Med. Arts Bldg., Houston 2, Tex.
Grignolo, Antonio, Clinica Oculistica della Univezsita di Parama, Italy.
Guerry, duPont III, 2015 Monument Ave., Richmond 19, Va.
Guibor, George P., 30 N. Michigan Ave., Chicago 2, Ill.
Gundersen, Trygve, 101 Bay State Rd., Boston 15, Mass.
Gunzburg, Julian, 193 N. Robertson Blvd., Beverly Hills, Calif.
Guth, Sylvester K., Lamp Div., General Elec. Co., Nela Park, Cleveland, Ohio.
Guy, Loren P., 40 E. 62nd St., New York, N.Y.
Guyton, Jack S., Henry Ford Hosp., Detroit 2, Mich.

H

Haas, Joseph S., 111 N. Wabash Ave., Chicago, Ill.
Haessler, F. Herbert, 723 E. Juniper Lane, Milwaukee 3, Wis.
Haik, George M., 812 Maison Blanche Bldg., New Orleans, La.
Hall, Thomas G., 516 Sutter St., San Francisco 2, Calif.
Hallett, Joseph W., Med. Arts Bldg., 16th & Walnut Sts., Philadelphia, Pa.
Hallum, Alton V., 326 Doctors Bldg., 490 Peachtree St., N.E., Atlanta 3, Ga.
Haney, Lawrence O., 100 E. St. Vrain St., Colorado Springs, Colo.
Hardesty, Hiram H., 10515 Carnegie Ave., Cleveland 6, Ohio.
Hare, James, 10515 Carnegie Ave., Cleveland 6, Ohio.
Hare, Robert, 416 N. Bedford Dr., Beverly Hills, Calif.
Harger, Robert Wm., 804 Hume-Mansur Bldg., Indianapolis 4, Ind.
Harley, Halvor L., 101 S. Indiana Ave., Atlantic City, N.J.
Harley, Robison D., 101 S. Indiana Ave., Atlantic City, N.J.
Harrington, David O., 384 Post St., Union Square, San Francisco 8, Calif.
Harris, John E., Dept. of Ophth., Univ. Ore. Med. Sch., Portland, Ore.
Hart, Clinton E., 2706 Cherry St., Bakersfield, Calif.
Hart, Wm. M., 5720 Ogden Rd., Washington 16, D.C.
Hartenbower, G. E., 410 Unity Bldg., Bloomington, Ill.
Hartshorne, Isaac, 30 W. 59th St., New York 19, N.Y.
Harvey, Elmer F., 942 Hanna Bldg., Cleveland 15, Ohio.
Havener, Wm. Henry, Univ. Hosp., Eye Clinic, Columbus, Ohio.
Hayden, Peter M., 950 E. 59th St., Chicago, Ill.
Heare, Louis C., Box 312, Port Arthur, Tex.
Heath, Gordon G., Sch. of Optometry, Univ. Calif., Berkeley 4, Calif.
Heath, Parker, 243 Charles St., Boston 14, Mass.
Hebbard, Frederick W., 153 Wallace Ave., Vallejo, Calif.
Heitger, Jos. D., 701 Heyburn Bldg., Louisville, Ky.
Henderson, John W., Univ. Hosp., Ann Arbor, Mich.
Henderson, John W., 102 2nd Ave., S.W., Rochester, Minn.
Henderson, Lawrence E., 502 Electric Bldg., Watertown, N.Y.
Henry, Margaret, 384 Post St., San Francisco 8, Calif.
Henry, Marvin D., 7141 S. Jeffery Ave., Chicago 49, Ill.
Hester, Marion W., 1255 Lakeland Hills Blvd., Lakeland, Fla.
Hilding, Anderson C., Research Lab., St. Luke's Hosp. Duluth 11, Minn.
Hildreth, H. Rommel, Beaumont Med. Bldg., 3720 Washington Blvd., St. Louis 8, Mo.
Hilgartner, H. L., 202 W. 13th St., Austin, Tex.
Hill, Howard F., Professional Bldg., 33 College Ave., Waterville, Me.
Hill, John Mounce, 108 Bay State Rd., Boston, Mass.
Hill, Robert V., 1329 Broadway, Longview, Wash.
Hirsch, Monroe J., P.O. Box 367, Ojai, Calif.
Hoffman, Franklin D., 550 Grant St., Pittsburgh 19, Pa.
Hoffman, Parker M., 134 E. 1st St., Corning, N.Y.
Hofstetter, Henry Wm., Div. of Optometry, Ind. Univ. Bloomington, Ind.
Hogan, Michael J., 450 Sutter St., San Francisco, Calif.
Hogenson, Clifford D., 308 Baker Bldg., Walla Walla, Wash.
Holmes, Dorothy B., 1816 R St., N.W., Washington 9, D.C.
Holmes, Wm. J., 45 Alexander Young Bldg., Honolulu 9, T.H.
Holstein, Theodore, 411 Medico-Dental Bldg., Sacramento 14, Calif.
Holt, Lawrence B., 209 Reynolds Bldg., Winston-Salem 3, N.C.
Holzer, Wm. F., 36 Pleasant St., Worcester 8, Mass.
Hooper, Robert J., 416 Bldg., 12th St. at 5th Ave., Columbus, Ga.
Horwich, Harry, 117 Yorkshire Dr., Biloxi, Miss.
Hosford, George N., 450 Sutter St., San Francisco, Calif.
Howard, Wm. M., 389 Linwood Ave., Buffalo 9, N.Y.
Howell, Homer P., 3022 E. 14th St., Oakland 1, Calif.
Hughes, Wm. F., Jr., 30 N. Michigan Ave., Chicago 2, Ill.
Hultgen, Wm. J., 7809 Herschel Ave., La Jolla, Calif.

Hurwitz, Paul, 55 E. Washington St., Chicago 2, Ill.

I

Imus, Henry A., 2828 Connecticut Ave., N.W., Apt. 913, Washington, D.C.
Ingalls, Raymond G., 57 Church St., Berlin, N.H.
Insel, Herman H., 770 Fidelity Med. Bldg., Dayton 2, Ohio.
Irvine, A. Ray, 9730 Wilshire Blvd., Beverly Hills, Calif.
Irvine, Rodman, 9730 Wilshire Blvd., Beverly Hills, Calif.
Irvine, Wendell C., 9730 Wilshire Blvd., Beverly Hills, Calif.
Israel, Milton R., 8230 Forsyth Blvd., Clayton 24, Mo.

J

Jacoby, M. W., 1364 Hanna Bldg., Cleveland 15, Ohio.
Jakobowits, Rafael, 655 Sutter Street, San Francisco 2, Calif.
Jakus, Marie A., Retina Foundation, 30 Chambers St., Boston 14, Mass.
James, Wm. M., 3720 Washington Blvd., Saint Louis 8, Mo.
Jampolsky, Arthur J., 2400 Clay St., San Francisco 15, Calif.
Jensen, Carl D. F., Medical-Dental Bldg., Seattle 1, Wash.
Johnson, Hobard C., 839 Belgrade Ave., Mankato, Minn.
Johnson, Lorand V., Carnegie Medical Bldg., 10515 Carnegie Ave., Cleveland 6, Ohio.
Johnson, Samuel B., 748 Oak St., Jackson, Miss.
Jones, W. Yerby, 436 Sycamore St., Buffalo, N.Y.
Jones, Wm. M., Dept. of Surgery, Univ. of Chicago, Chicago, Ill.
Judd, John H., 1020 Med. Arts Bldg., Omaha, Neb.

K

Kadin, Maurice, 535 Main St., Racine, Wis.
Kahan, Edmund, 400 W. End Ave., New York, N.Y.
Kahler, Arthur R., 2615 Capitol Ave., Sacramento 16, Calif.
Kantar, Bruce L., 2925 Dean Blvd., Minneapolis 16, Minn.
Kara, Gerald B., 70 Park Ave., New York 16, N.Y.
Katz, Dewey, 99 Pratt St., Hartford 3, Conn.
Katz, I. Herbert, 713 E. Genesee St., Syracuse 2, N.Y.
Katzin, Herbert M., 11 E. 68th St., New York, N.Y.
Kellerman, Leo D., 61-34 188th St., Flushing, N.Y.
Kennedy, Roscoe J., Cleveland Clinic, 2020 E. 93rd St., Cleveland 6, Ohio.
Kent, Louis R., 104 W. Clark St., Champaign, Ill.
Key, Samuel N., Jr., 120 W. 7th St., Austin, Tex.
Keyes, John E. L., 613 Home Savings & Loan Bldg., Youngstown 3, Ohio.
Kimura, Samuel J., Univ. Calif. Hosp., San Francisco, Calif.
King, George L., 537 E. Market St., Alliance, Ohio.
King, John H., Jr., 4116 Blackthorn St., Chevy Chase 15, Md.
Kinsey, V. Everett, 690 Mullett St., Kresge Institute, Detroit 26, Mich.
Kirk, Robert C., 15644 Madison Ave., Lakewood 7, Ohio.
Kirkland, Theo N., Jr., Box 1203, Foley, Ala.
Klien, Bertha A., Univ. Chicago, Chicago 37, Ill.
Knapp, Arnold, 24 W. 55th St., New York 19, N.Y.
Knapp, Arthur A., 907 5th Ave., New York 21, N.Y.
Knapp, Phillip, 635 W. 165th St., New York 32, N.Y.
Knighton, Willis S., 121 E. 61st St., New York, N.Y.
Knox, George W., Sch. of Optometry, Ohio State U., Columbus, Ohio.
Koch, F. L. Philip, 9 Tanglewylde Ave., Bronxville 8, N.Y.
Koenig, Harry, 623 Michigan St., Ishpeming, Mich.
Koenig, Ivan J., 40 North St., Buffalo 2, N.Y.
Koistinen, Aune M., Univ. Eye Clinic of Helsinki, Helsinki, Finland.
Kornzweig, Abr. L., 1200 5th Ave., New York, N.Y.
Kottler, Saul, 831 Guardian Bldg., Cleveland 14, Ohio.
Krause, Arlington C., 950 E. 59th St., Chicago, Ill.
Krewson, Wm. E., III, 1930 Chestnut St., Philadelphia 3, Pa.
Kronenberg, Bernard, 737 Park Ave., New York, N.Y.
Kronfeld, Peter C., 111 N. Wabash Ave., Chicago 2, Ill.
Krug, Joseph H. 988 5th Ave., New York 21, N.Y.
Kuhn, Hedwig S., 60 Glendale Park, Hammond, Ind.
Kummer, Wm. M., 1231 Lakeland Hills Blvd., Lakeland, Fla.

L

Lachman, George S., 450 Sutter St., San Francisco, Calif.
Land, Richard E., 811 Eastwood Ave., Chicago, Ill.
Lang, Louis R., 8641 Colesville Rd., Silver Springs, Md.
Lang, Richard E., 463 Passaic Ave., Passaic, N.J.
Larkin, Bernard J., 305 Hume-Mansur Bldg., Indianapolis 4, Ind.
Laval, Joseph, 136 E. 64th St., New York, N.Y.
Lawson, Lawrence J., Jr., 636 Church St., Evanston, Ill.
Leachman, John Wm., 400 N. Main St., Warsaw, N.Y.
Leckert, Edmund L., Jr., 1214 Nashville Ave., New Orleans, La.
Lee, Otis S., 311 Utica Square, Medical Center, Tulsa, Okla.
Legett, Carey, Jr., 1707 Colorado, Austin, Tex.
Leinfelder, P. J., Univ. Hosp., Iowa City, Iowa.
Lemoine, Albert N., 411 Alameda Rd., Country Club Plaza, Kansas City 2, Mo.
Lemoine, Albert N., Jr., 4620 J. C. Nichols Pkwy., Kansas City 12, Mo.
Leopold, Irving, H., 1930 Chestnut St., Philadelphia 3, Pa.
Lepard, C. W., 1025 David Whitney Bldg., Detroit 26, Mich.
Lerner, Macy L., 332 Park Ave., Rochester 7, N.Y.
Levitt, Herbert, 167 College, Toronto, Canada.
Levy, Abram, 106 E. Union Ave., Bound Brook, N.J.
LeWin, Thurber, 112 Linwood Ave., Buffalo, N.Y.
Lewis, Philip M., Exchange Bldg., Memphis 3, Tenn.
Lieberman, Howard L., 2800 Milwaukee Ave., Chicago 18,
Liebman, Sumner D., 115 Bay State Rd., Boston 15, Mass.
Lightfoot, Vernon F., 52 N. 1st East, Logan, Utah.
Linkaz, Arthur, 6 E. 76th St., New York 21, N.Y.
Linn, Jay G., Jr., 7075 Jenkins Arcade Bldg., Pittsburgh 22, Pa.
Linn, Jay G., Sr., 7075 Jenkins Arcade, Pittsburgh 22, Pa.
Lipetz, Leo E., Dept. of Ophth., Ohio State Univ., Columbus 10, Ohio.
Lipp, Frank E., City Natl. Bank Bldg., Suite 830, 16th and Harney Sts., Omaha 2, Neb.
Little, Milton F., 85 Jefferson St., Hartford, Conn.
Lloyd, Ralph I., 14 8th Ave., Brooklyn 17, N.Y.
Locke, John C., 1414 Drummond St., Montreal, Canada.
Lordan, John Paul, 133 S. Lasky Dr., Beverly Hills, Calif.
Loutfallah, Michel, 1826 State St., Santa Barbara, Calif.
Lowenstein, Otto, 865 Park Ave., New York, N.Y.
Luedde, Philip S., Hampton Village Med. Center, St. Louis 2, Mo.
Luhr, Alfred F., 40 North St., Buffalo, N.Y.
Luhr, John P., 40 North St., Buffalo, N.Y.
Lutman, Frank C., Germantown Prof. Bldg., Greene and Coulter Sts., Philadelphia 44, Pa.
Lyda, Wood, 706 36th Ave., Seattle, Wash.
Lyle, Donald J., 601 Union Trust Bldg., 4th and Walnut, Cincinnati 2, Ohio.

Mc

McAlester, A. W., III, 2003 Bryant Bldg., Kansas City, Mo.
McAlpine, Paul T., 129 Summit Ave., Summit, N.J.
McBain, Earle H., 1010 B. St., San Rafael, Calif.
McCallum, George C., 301 Eugene Med. Center, Eugene, Ore.
McCannel, Malcolm A., 325 Doctors Bldg., Minneapolis 2, Minn.
McCaslin, Murray F., 550 Grant St., Pittsburgh 19, Pa.
McClure, G. David, 565 Eastern Parkway, Louisville, Ky.
McCulloch, Clement, 830 Med. Arts Bldg., Toronto, Can.
McDonald, F. Robb, Lankenau Med. Bldg., Philadelphia, Pa.
McEwen, W. K., Univ. Calif. Med. Center, San Francisco, Calif.
McGarvey, W. E., 802 Jackson City Bank Bldg., Jackson, Mich.
McGavic, John S., 601 Montgomery Ave., Bryn Mawr, Pa.
McGowan, W. L., 510 Union Central Bldg., Cincinnati 2, Ohio.
McGraw, James L., 615 State Tower Bldg., Syracuse 4, N.Y.
McGuire, W. P., 105 N. Braddock St., Winchester, Va.
McKay, Edward D., 812 Fisk Bldg., Amarillo, Tex.
McKee, Joseph W., 1332 Professional Bldg., Kansas City, Mo.
McLean, John M., 525 E. 68th St., New York 21, N.Y.

M

MacDonald, Roy K., 421 Med. Arts Bldg., Toronto, Ontario, Canada.
MacLean, Angus L., 1201 N. Calvert St., Baltimore 2, Md.
Macnie, John P., 635 W. 165th St., New York 32, N.Y.

Madeley, H. Randall, 1727 Sonoma St., Vallejo, Calif.
Magee, George R., 1331 Hillside Dr., Reno, Nev.
Magnuson, Robert H., 150 E. Broad St., Columbus, Ohio.
Maier, Paul, 723 Congress St., Portland, Me.
Makley, Torrence A., 21 E. State St., Columbus, Ohio
Mallek, Howard, 924 Vancouver Block, Vancouver, B.C.
Mamelok, Alfred E., 628 E. 20th St., New York 9, N.Y.
Manchester, Paul T., Jr., 478 Peachtree St., N.E., Atlanta, Ga.
Mandelbaum, Joseph, 101 Lafayette Ave., Brooklyn 17, N.Y.
Mann, W. A., 30 N. Michigan Ave., Chicago 2, Ill.
Mannis, Aaron A., 109 N. Wabash Ave., Chicago, Ill.
Marcus, Arthur A., 30 E. 40th St., New York, N.Y.
Marg, Elwin, Sch. of Optometry, Univ. Calif., Berkeley 4, Calif.
Markley, Richard E., 520 Mayer Bldg., Portland 5, Ore.
Marlow, Searle B., 1025 State Tower Bldg., Syracuse 2, N.Y.
Marshall, M. R., North Pavilion, Univ. Hosp., Edmonton, Canada.
Martens, Theodore G., 102-110 2nd Ave., S.W., Rochester, Minn.
Martz, George E., 608 N. 3rd St., Harrisburg, Pa.
Masters, Robert J., 805 Hume Mansur Bldg., Indianapolis 4, Ind.
Matthews, John L., 929 Nix Prof. Bldg., San Antonio 5, Tex.
Mattis, Robert D., 634 N. Grand Blvd., St. Louis 3, Mo.
Matusak, Lucian R., 5605 W. Belmont Ave., Chicago 34, Ill.
Maumenee, Alfred E., Johns Hopkins Hosp., Baltimore 5, Md.
Maynard, Robert E., 4303 Locust St., Philadelphia 4, Pa.
Meisenbach, Albert E., Jr., 317 Med. Arts Bldg., Dallas, Tex.
Melton, Carlton E., Jr., Dept. of Physiology, Southwestern Med. Sch., 2211 Oaklawn Ave., Dallas, Tex.
Merriam, George R., Jr., 635 W. 165th St., New York 32, N.Y.
Mesirow, M. E., 117 E. Cook St., Santa Maria, Calif.
Meyer, Dexter, Jr., 508 N. Grand Blvd., St. Louis, Mo.
Meyer, George P., 410 Haddon Ave., Camden, N.J.
Meyer, Samuel J., 109 N. Wabash, Chicago 2, Ill.
Middleton, J. Myron, 6515 Wilshire Blvd., Los Angeles, Calif.
Miles, Paul W., Lister Bldg., 4500 Olive St., St. Louis 10, Mo.
Miller, Claude K., Box 1627, 601 Douglas St., Wenatchee, Wash.
Miller, Geo. Leslie, 14805 Detroit Ave., Lakewood, Ohio.
Miller, Miriam, 350 Post St., San Francisco 8, Calif.
Mills, Dawson A., Recent address unknown.
Mills, Lloyd, Recent address unknown.
Mims, James L., Jr., Med. Arts Bldg., San Antonio, Tex.
Minsky, Henry, 960 Park Ave., New York, N.Y.
Mintz, Maxwell A., 497 Glen St., Glens Falls, N.Y.
Mohney, Glenn E., Med. Bldg., 311 Pine St., Port Huron, Mich.
Monahan, Robert Hugh, 1023 Lowry Med. Arts Bldg., St. Paul, Minn.
Morgan, James C., Jr., West Point, Ga.
Morgan, Meredith W., Jr., 2406 Tulure Ave., El Cerrito 9, Calif.
Morgana, Dante J., 13 Main St., Lockport, N.Y.
Morris, Jean W., Johnston Bldg., Muncie, Ind.
Morrison, W. Howard, 1500 Med. Arts Bldg., Omaha, Neb.
Moses, Robert A., 1101½ S. 3rd St., Delavan, Wis.
Motto, Paul, 650 Rose Bldg., Cleveland 15, Ohio.
Mullen, Carroll R., 2025 Locust St., Philadelphia 3, Pa.
Murray, Edward S., 1 Shattuck St., Boston, Mass.
Myers, Roscoe W., 36 Pleasant St., Worcester 8, Mass.

N

Nadbath, Rudolph P., Recent address unknown.
Naples, Ange S., 570 Delaware Ave., Buffalo 2, N.Y.
Naquin, Howard, Johns Hopkins Hosp., Baltimore 5, Md.
Nelson, Robert C., 1902 Union Central Bldg., Cincinnati, Ohio.
Nethercut, Glenway W., 25 E. Washington St., Chicago, Ill.
Newell, Frank W., 950 E. 59th St., Chicago, Ill.
Nicholl, Russell J., 10515 Carnegie Ave., Cleveland 6, Ohio.
Noble, Bertha R., 1636 Toledano St., New Orleans 12, La.
Noe, Carl A., 601 Higley Bldg., Cedar Rapids, Iowa
Norene, Robert A., 3741 Stocker St., Los Angeles 14, Calif.
Norton, Herman J., Jr., Recent address unknown.
Nosik, W. A., 10515 Carnegie Ave., Cleveland, Ohio.
Novak, Joseph F., 520 Med. Arts Bldg., Pittsburgh 13, Pa.
Nugent, Maurice W., 2010 Wilshire Blvd., Los Angeles 57, Calif.

O

Oaks, Lewis W., 677 N. University Ave., Provo, Utah.
O'Brien, Cecil S., 43 E. Jackson St., Tucson, Ariz.
O'Brien, James M., 735 Clinton Ave., Bridgeport, Conn.
Odom, Robert E., 331 Haywood Bldg., Asheville, N.C.
Ogle, Kenneth N., Mayo Clinic, Rochester, Minn.
Ohly, John H., 22 Schermerhorn St., Brooklyn, N.Y.
O'Kane, Thomas W., 830 Lowry Med. Arts Bldg., St. Paul, Minn.
Olmsted, K. Elizabeth Pierce, 568 Lafayette Ave., Buffalo, N.Y.
Ormsby, Hugh L., Banting Institute, Toronto 17, Ontario, Canada.
O'Rourke, Donald H., 920 Republic Bldg., Denver, Colo.
O'Rourke, James F., Nat. Institute of Health, Bethesda, Md.
Osler, Jay K., 5 Grove St., Bangor, Me.
Owens, W. C., 102 N. 8th St., Allentown, Pa.
Ozment, Thomas L., 2287 E. 39th St., Tulsa 3, Okla.

P

Palmer, Milton A., 18 Park Blvd., Lancaster, N.Y.
Palmerton, Ernest S., Park Region Med. Center, Fergus Falls, Minn.
Pang, Herbert G., 52 S. Vineyard St., Honolulu, T.H.
Paton, R. Townley, 927 Park Ave., New York, N.Y.
Patterson, John W., 2109 Adelbert Rd., Cleveland 6, Ohio.
Patz, Arnall, 920 St. Paul St., Baltimore 2, Md.
Paul, Thomas O., 2205 Highland Ave., Birmingham 5, Ala.
Paulson, Hubert O., 508 Sharp Bldg., Lincoln 8, Neb.
Payne, Brittain F., 17 E. 72nd St., New York 21, N.Y.
Pearlman, Maurice D., 904 W. Adams St., Chicago 7, Ill.
Perera, Charles A., 70 E. 66th St., New York, N.Y.
Peter, Philip A., 1117 Minor Ave., Seattle 1, Wash.
Pettit, W. A., 727 W. Seventh St., Los Angeles, Calif.
Pfingst, Harry A., 1015 Heyburn Bldg., Louisville 2, Ky.
Phillips, Josephine D., 2010 E. 102nd St., Cleveland 6, Ohio.
Pichette, Henri, 425 St. John St., Quebec City, Quebec, Canada
Pinkoson, Charles, 826 W. University Ave., Gainesville, Fla.
Pischel, Dohrmann K., 490 Post St., San Francisco 2, Calif.
Polan, Charles M., 1042 6th Ave., Huntington, W.Va.
Posner, Adolph, 667 Madison Ave., New York 21, N.Y.
Post, Lawrence T., 508 N. Grand Blvd., St. Louis 3, Mo.
Post, Martin H., Jr., 508 N. Grand Blvd., St. Louis 3, Mo.
Potter, Wm. B., 521 22nd St., Galveston, Tex.
Potts, Albert M., 2065 Adelbert Rd., Cleveland 6, Ohio.
Praglin, Julius, 3868 Carnegie Ave., Cleveland, Ohio.
Prince, Jack H., Dept. of Ophthal., Univ. Hosp., Columbus, Ohio.
Pritikin, Roland I., 1211 Talcott Bldg., Rockford, Ill.
Puntenney, Irving, 720 N. Michigan Ave., Chicago, Ill.
Pushkin, Edward A., 122 S. Michigan Ave., Chicago, Ill.

Q

Quilala, Francisco V., V. Luna Gen. Hosp., Quezon City, Philippines.

R

Raffo, Julio C., Gremios 465, Lima, Peru, S.A.
Ralph, Fenn. T., 50 E. 72nd St., New York 21, N.Y.
Rasgorshek, Robert H., 425 Aquila Ct., Omaha, Neb.
Ravdin, Emile M., 239 S. La Cienega, Beverly Hills, Calif.
Reeder, James E., Jr., 523 Davidson Bldg., Sioux City 13, Iowa.
Reeh, Merrill J., 919 S. W. Taylor St., Bldg., Portland 5, Ore.
Reese, Algernon B., 73 E. 71st St., New York 21, N.Y.

Reese, Warren S., 2118 Locust St., Philadelphia 3, Pa.
Regan, James J., 520 Commonwealth Ave., Boston 15, Mass.
Reid, Frederick K., 500 N. George St., Rome, N.Y.
Reid, Horace W., 225 Union Central Bldg., Cincinnati 2, Ohio.
Reitz, Herbert R., 446 Linwood Ave., Buffalo, N.Y.
Riddle, Ransford J., 14 Porter Way, Sharon, Pa.
Riffenburgh, Ralph S., 615 1 Trust Bldg., 595 E. Colorado St., Pasadena 1, Calif.
Rigg, James P., 521 Road Ave., Grand Junction, Colo.
Robbins, Alfred R., 1930 Wilshire Blvd., Los Angeles 5, Calif.
Roberts, Walter L., 8225 Ramsgate, Los Angeles, Calif.
Robison, James T., Jr., 4620 Nichols Pkwy., Kansas City 12, Mo.
Rodin, Frank H., 490 Post St., San Francisco 2, Calif.
de Roetth, Andrew F. M., 1006 Overbluff, Spokane, Wash.
de Roetth, Andrew, Jr., 622 W. 168th St., New York 32, N.Y.
Romaine, Hunter, 111 E. 65th St., New York 21, N.Y.
Rones, Benjamin, 1200 18th St., N.W., Washington, D.C.
Roper, Kenneth L., 55 E. Washington St., Chicago 2, Ill.
Rosen, Emanuel P., 692 High St., Newark, N.J.
Rosenbaum, Harry D., 3720 Washington Blvd., St. Louis, Mo.
Rosenberg, Abner S., 142 Joralemon St., Brooklyn 2, N.Y.
Rosenberg, Alan J., 450 Sutter St., San Francisco, Calif.
Rosenthal, Benj. C., 41 Eastern Pkwy., Brooklyn 38, N.Y.
Rosenthal, J. Wm., 3615 Prytania St., New Orleans 15, La.
Rosner, Robert S., Rose Bldg., Cleveland 6, Ohio.
Ross, Cecelia, 1318 2nd St., Santa Monica, Calif.
Ross, Joseph V. M., 321 E. Front St., Berwick, Pa.
Rouse, David M., 117 E. Monroe, Mexico, Mo.
Rowland, Louise S., Wilmer Inst., Johns Hopkins Hosp., Baltimore, Md.
Rubin, Milton R., 1331 Wishon Ave., Fresno, Calif.
Rucker, C. Wilbur, Mayo Clinic, Rochester, Minn.
Ruedemann, A. D., 1633 David Whitney Bldg., Detroit 26, Mich.
Ruedemann, Albert D., Jr., 1633 David Whitney Bldg., Detroit, Mich.
Ruth, Paul E., 510 Dollar Bank Bldg., Youngstown, Ohio.
Rutherford, Cyrus W., 4601 N. Pennsylvania St., Indianapolis, Ind.
Ryan, Edward P., 153 Warren St., Glens Falls, N.Y.
Ryan, Ralph W., 158 High St., Morgantown, W.Va.
Ryan, Wm. F., 761 Washington St., Norwood, Mass.
Rychener, Ralph O., 130 Madison Ave., Memphis 3, Tenn.

S

Sacks-Wilner, Erwin, 225 W. State St., Trenton, N.J.
Sakler, Barnet R., Doctors Bldg., Cincinnati 2, Ohio.
Samuels, Bernard, 57 W. 57th St., New York, N.Y.
Sanders, T. E., 3720 Washington Blvd., St. Louis 8, Mo.
Sands, Abraham M., 874 Carroll St., Brooklyn 15, N.Y.
Sarnat, Leonard A., 363 Park Ave., Glencoe, Ill.
Sauer, John J., 30 E. 40th St., New York, N.Y.
Scarney, Herman D., 573 Fisher Bldg., Detroit 2, Mich.
Schachat, Walter S., 39 E. 75th St., New York, N.Y.
Schachne, Lewis, 140 Wadsworth Ave., New York 33, N.Y.
Scheffler, Milton M., 55 E. Washington St., Chicago 2, Ill.
Scheie, Harold G., 313 S. 17th St., Philadelphia 3, Pa.
Schepens, Charles L., 243 Charles St., Boston, Mass.
Schlaegel, Theodore F., Jr., Indiana Univ. Med. Center, Indianapolis, Ind.
Schlossman, Abraham, 667 Madison Ave., New York 21, N.Y.
Schmerl, Ernst, 2805 Oatis Ave., Toledo 6, Ohio.
Schoel, Robert E., 211 S. Saratoga Ave., New Orleans 12, La.
Scholz, Roy O., 11 E. Chase St., Baltimore 2, Md.
Schonberg, Albert L., 32 Med. Arts Sq., Albuquerque, N.M.
Schopp, Robert C., 49 Linwood Ave., Buffalo 9, N.Y.
Schrimpf, Cyril E., 2500 Clifton Ave., Cincinnati 19, Ohio.
Schultz, Jacob F., 1304 Walker Ave., Houston, Tex.
Schuster, Stephan A., 1st Nat. Bank Bldg., El Paso, Tex.
Schwartz, Bernard, Univ. Hosp., Iowa City, Iowa
Schwartz, Frederick O., 508 N. Grand Blvd., St. Louis 3, Mo.
Schwartz, Virgil J., 312 Doctor's Bldg., Minneapolis, Minn.
Schwarz, Gerald T., 10515 Carnegie Ave., Cleveland 6, Ohio.
Sezer, F. N., Cerrahpasa Hospital, Istanbul, Turkey.
Shaad, Dorothy J., 411 Nichols Rd., Kansas City, Mo.
Shafer, Donald M., 140 E. 54 St., New York, N.Y.
Shaffer, Robert N., 490 Post St., San Francisco 2, Calif.
Shahan, Philip T., 508 N. Grand Ave., St. Louis 3, Mo.
Shahan, Wm. E., 508 N. Grand Ave., St. Louis 3, Mo.
Shapira, Theodore M., 109 N. Wabash Ave., Chicago 2, Ill.
Shapiro, Ernest K., Recent address unknown.
Sharbaugh, George B., 25 Prospect St., Trenton 8, N.J.
Sheard, Charles, P.O. Box 543, Rochester, Minn.
Sheldon, Maurice G., 604-606 Exchange Nat'l. Bank Bldg., Olean, N.Y.
Sheppard, L. Benjamin, 301 Med. Arts Bldg., Richmond 19, Va.
Sherman, A. Russell, 15 Washington St., Newark, N.J.
Sherman, Elbert S., 15 Washington St., Newark, N.J.
Shoch, David E., 700 N. Michigan Ave., Chicago, Ill.
Shusterman, Morris, 345 Bloor St., W., Toronto 5, Ontario, Canada.
Siegel, Edward, 81 Brinkerhoff St., Plattsburg, N.Y.
Silverstein, Arthur L., 16 Crescent Ave., Passaic, N.J.
Simonton, John T., Bland Brook Lodge, Rye, N.Y.
Sitchevska, Olga, 30 5th Ave., New York, N.Y.
Sitney, Julian J., 87 N. 6th St., San Jose 12, Calif.
Skow, John D., 2001 Collingwood Ave., Toledo 2, Ohio.
Skowron, John J., 4810 N. Milwaukee Ave., Chicago 30, Ill.
Sloan, Henry L., 106 W. 7th St., Charlotte 2, N.C.
Smallen, Benjamin, 345 Linwood Ave., Buffalo 9, N.Y.
Smelser, George K., 630 W. 168th St., New York, N.Y.
Smillie, John W., Dept. of Ophth., Univ. Hosp., Ann Arbor, Mich.
Smith, Byron, 722 Park Ave., New York, N.Y.
Smith, Hiram J., Recent address unknown.
Smith, Homer E., 508 E. S. Temple St., Salt Lake City, Utah.
Smith, James W., 1016 5th Ave., New York 28, N.Y.
Smith, Jaroud B., Jr., 1006 Church St., Alva, Okla.
Smith, Joseph G., 450 Sutter St., San Francisco 28, Calif.
Smithson, Robert A., Jr., 1530 Arizona Ave., Santa Monica, Calif.
Snell, Albert C., Jr., Strong Memorial Hosp., Rochester, N.Y.
Snydacker, Daniel, 109 N. Wabash Ave., Chicago 2, Ill.
Snyder, John C., 1 Shattuck St., Boston, Mass.
Snyder, M. Wilson, 14 Porter Way, Sharon, Pa.
Snyder, Stuart S., 525 E. 68th St., New York 21, N.Y.
Soper, Gail R., 636 Church St., Evanston, Ill.
Sovik, Wm. E., 203 Mahoning Bank Bldg., Youngstown, Ohio.
Spaeth, Edmund B., 1930 Cheatnut St., Philadelphia, Pa.
Stauffer, Harry B., 234 Madison St., Jefferson City, Mo.
Steinberg, Abraam, 323 Jenkins Bldg., Pittsburgh 22, Pa.
Steinberg, Bernhard, 2805 Oatis Ave., Toledo 6, Ohio.
Steinberg, Theodore, Patterson Bldg., Fresno, Calif.
Stephens, H. Frederick, 195 Thayer St., Providence 6, R.I.
Stevens, Ralph W., Baker Bldg., Walla Walla, Wash.
Stillerman, Manuel L., 109 N. Wabash Ave., Chicago 2, Ill.
Stine, George T., 327 E. State St., Columbus 16, Ohio.
Stoddard, Kenneth B., Sch. of Optometry, Univ. of Calif., Berkeley, Calif.
Stokes, J. Jack, 384 Peachtree St., N.E., Atlanta 3, Ga.
Stokes, Wm. H., Lake City, Mich.
Stolzar, Irwin H., Travis Clinic, Jacksonville, Tex.
Stone, Leon S., Dept. of Anatomy, Yale Univ. Sch. of Med., New Haven, Conn.
Stone, Wm. Jr., 1101 Beacon St., Brookline, Mass.
Streicher, Carl J., 217 Park Pl., Ashtabula, Ohio.
Sugar, H. Saul, 18140 San Juan, Detroit 26, Mich.
Suie, Ted, Dept. Ophth., Ohio State Univ., Columbus, Ohio.

Sullivan, Charles T., 797 Elmwood Ave., Rochester 20, N.Y.
Sullivan, John V., 109 N. Crawford Ave., Carroll, Iowa.
Suran, Anita A., Dept. of Ophth., Univ. Calif. Med. Center, San Francisco, Calif.
Swab, Charles M., 1316 Med. Arts Bldg., Omaha, Neb.
Swan, Kenneth C., Univ. Oregon Med. Sch., Portland, Ore.
Swiontkowski, Stanley D., 4341 S. Archer Ave., Chicago 32, Ill.
Sykowski, Peter S., 1330 Union St., Schenectady 8, N.Y.

T

Tabb, W. Granville, 403 Doctors Bldg., Atlanta, Ga.
Tait, Edwin F., 1324 W. Main St., Norristown, Pa.
Tanner, Owen R., 300 Homer Ave., Palo Alto, Calif.
Tassman, Isaac S., 711 Med. Arts Bldg., Philadelphia, Pa.
Taylor, E. Merle, 1020 S. W. Taylor, Portland, Ore.
Taylor, Frank W., Ohio State Univ. Med. Center, Columbus, Ohio.
Taylor, Israel B., 216 Harley Chambers, Jeppe St., Johannesburg, South Africa.
Teed, Roy Wm., V. A. Hosp., Dayton, Ohio
Theobald, G. D., 715 Lake St., Oak Park, Ill.
Theodore, Frederick H., 667 Madison Ave., New York 21, N.Y.
Thigpen, Charles A., 401 S. Court St., Montgomery 5, Ala.
Thomas, Charles I., 10515 Carnegie Ave., Cleveland 6, Ohio.
Thomas, Edward R., 60 Wyoming St., Dayton, Ohio.
Thomas, John H., 262 E. Market St., Warren, Ohio.
Thomas, Maxwell, 206 Med. Arts Bldg., Dallas, Tex.
Thompson, Edward H., 19 W. 7th St., Cincinnati, Ohio.
Thornburgh, Robert G., 117 E. 8th St., Long Beach 13, Calif.
Thorpe, Harvey E., 323 Jenkins Arcade, Pittsburgh 22, Pa.
Thygeson, Phillips, 222 Meridian Rd., San Jose, Calif.
Tinkness, Donald E., Ituri Towers, Greenwich, Conn.
Tour, Robert L., 384 Post St., San Francisco 8, Calif.
Tower, Paul, 2007 Wilshire Blvd., Los Angeles 57, Calif.
Tracht, Robert R., 404 Lowry Med. Arts Bldg., St. Paul 2, Minn.
Trapp, Claude W., Jr., 640 S. Kingshighway, St. Louis 10, Mo.
Troutman, Richard C., 430 E. 63rd St., New York 21, N.Y.
Trowbridge, Dwight H., Jr., 2900 Fresno St. Bldg., Fresno, Calif.
True, William Ray, 506 Professional Bldg., Monterey, Calif.
Trueman, Robert H., 1917 Panama St., Philadelphia 3, Pa.
Truhlsen, Stanley M., 1500 Med. Arts Bldg., Omaha, Neb.
Turtz, Charles A., 65 Central Park, W., New York, N.Y.
Tusak, Ervin A., 115 E. 90th St., New York 28, N.Y.
Tyner, George S., Metropolitan Bldg., Denver, Colo.

U

Unruh, John W., 303 Pacific Bldg., Roseburg, Ore.
Unsworth, Arthur C., 85 Jefferson St., Hartford, Conn.
Urrets-Zavalia, Alberto, Jr., Casillo de Correo 301, Cordoba, Argentina

V

Vail, Derrick T., 700 N. Michigan Ave., Chicago 11, Ill.
Van Heuven, J. Alexander, 244 Edwards St., New Haven, Conn.
Van Wien, Stefan, 104 S. Michigan Blvd., Chicago 3, Ill.
Vanzant, Thomas J., 1403 Hermann Prof. Bldg., Houston, Tex.
Varga, Laszlo, 30 Chambers St., Boston, Mass.
Vaughan, Daniel G., Jr., 220 Meridian, San Jose, Calif.
Vaughan, James B., 129 Delaware Rd., Kenmore, N.Y.
Veirs, Everett R., Scott and White Clinic, Temple, Tex.
Velarde, Herminio, Sr., Phil. Gen. Hosp., Manila, P.I.
Venable, Howard P., 11 N. Jefferson, St. Louis, Mo.
Verhoeff, F. H., 395 Commonwealth Ave., Boston 15, Mass.
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Smith, James W.
Snyder, Stuart S.
Theodore, Frederick
Troutman, Richard C.
Turtz, Charles A.
Tusak, Ervin A.
VonSallman, Ludwig
Webster, David H.
Wheeler, Maynard G.
Wise, George N.

NIAGARA FALLS
Goetzman, Arthur C.

OLEAN
Sheldon, Maurice G.

PLATTSBURG
Siegel, Edward

ROCHESTER
Caccamise, William C.
Gipner, John F.
Lerner, Macy L.
Norton, Herman J., Jr.
Snell, Albert C., Jr.
Sullivan, Charles T.

ROME
Reid, Frederick K.

RYE
Simonton, John T.

SARATOGA SPRINGS
Goodfellow, Thomas J.

SCHENECTADY
Sykowski, Peter S.

SYRACUSE
Gillette, David F.
Katz, I. Herbert
McGraw, James L.
Marlow, Searle B.

VALLEY COTTAGE
Cumming, Edith L. Wentworth

WARSAW
Leachman, John W.

WATERTOWN
Atkinson, Walter S.
Henderson, Lawrence E.

WEST ISLIP
Bergmann, Robert B.

NORTH CAROLINA

ASHVILLE
Odom, Robert E.

CHARLOTTE
Sloan, Henry L.

DURHAM
Anderson, Banks

GOLDSBORO
Bizzell, James W.

WINSTON-SALEM
Holt, Lawrence B.

OHIO

ALLIANCE
King, George L.

ASHTABULA
Streicher, Carl J.

CINCINNATI
Abrahanson, Ira A.
Ascher, Charles K. W.
Brown, Albert L.
Lyle, Donald J.
McGowan, William L.
Reid, Horace W.

Sakler, Barnet R.
Schrimpf, Cyril E.
Thompson, Edward H.

CLEVELAND
Ballintine, Elmer J.
Bell, Richard P., Jr.
Brandwan, Samuel R.
Bruner, Abram B.
Budd, Francis X.
Chamberlain, W. P., Jr.
Chickering, Donald H.
Gans, Jerome A.
Gans, Morris E.
Gilger, Anita P.
Guth, Sylvester K.
Hardesty, Hiram H.
Hare, James
Harvey, Elmer F.
Jacoby, Marl. W.
Johnson, Lorand V.
Kennedy, Roscoe J.
Kirk, Robert C.
Kottler, Saul
Motto, Paul
Nichol, Russell J.
Nosik, William A.
Patterson, John W.
Phillips, Josephine D.
Potts, Albert M.
Praglin, Julius
Rosner, Robert S.
Schwarz, Gerald T.
Thomas, Charles I.
Waitzman, Morton B.
Wolpaw, Benjamin J.

CLEVELAND HEIGHTS
Nelson, Robert C.

COLUMBUS
Battles, Morris L.
Brecher, Gerhard A.
Ellerbrock, Vincent J.
Fry, Glenn A.
Havener, Wm. Henry
Knox, George W.
Lipetz, Leo E.
Magnuson, Robert H.
Makley, Torrence A.
Prince, Jack H.
Stine, George T.
Suic, Ted
Taylor, Frank W.

DAYTON
Insel, Herman H.
Teed, Roy Wm.
Thomas, Edward R.

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Ellenberger, Carl
Miller, George L.

NEWBURY
Frohring, William O.

PARMA
Graham, John H.

TOLEDO
Schmerl, Ernest
Skow, John D.
Steinberg, Bernhard
Vesey, Frank A.
Winger, Ira B.

UNIVERSITY HEIGHTS
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Thomas, John H.

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Bloomberg, Louis
Keyes, John E. L.
Evans, William H.
Ruth, Paul E.
Sovik, Wm. E.

OKLAHOMA

NORMAN
Wiley, George A.

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Lee, Otis S.
Ozment, Thomas L.

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Flanagan, Roger M.

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McCallum, George C.

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Christensen, Leonard
Gehrsitz, Leta B.
Harris, John E.
Markley, Richard E.
Reeh, Merril J.
Swan, Kenneth C.
Taylor, E. Merle
Unruh, John W.

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ARDMORE
Vogel, Adolph W.

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Duane, Thomas D.

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Covey, John K.

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Alberstadt, Norbert F.

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Tait, Edwin F.

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Askovitz, Samuel I.
Blazar, Howard A.
Brav, Soloman S.
Carmichael, Paul L.
Cowan, Alfred
DeLong, Samuel L.
Ellis, Richard A.
Gettes, Bernard C.
Gowens, Harry L., Jr.
Green, Harry
Hallett, Joseph W.
Krewson, William E., III
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Lutman, Frank C.
McDonald, Philip R.
Maynard, Robert E.
Mullen, Carroll R.
Reese, Warren S.
Scheie, Harold G.
Spaeth, Edmund B.
Tassman, Isaac S.
Trueman, Robert H.
Waldman, Joseph
Weimar, Virginia L.
Zentmayer, William

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Davies, Robert H.
Evans, Thomas M.
Goldberg, Sol
Grana, Phillip C.
Hoffman, Franklin D.
Linn, Jay G., Jr.
Linn, Jay G., Sr.
McCaslin, Murray F.
Novak, Joseph F.
Steinberg, Abraam
Thorpe, Harvey E.
Weisser, Charles W.
Wolken, Jerome J.

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Bloomenthal, Sanford R.
Riddle, Ransford J.
Snyder, M. Wilson

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Benedict, Walter H., Jr.

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Rychener, Ralph O.

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Hilgartner, H. L.
Key, Samuel N., Jr.
Legett, Carey, Jr.

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Gill, Earl K.

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Schuster, Stephen A.

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Potter, Wm. Bentley

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Vanzant, Thomas J.

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Hill, Robert V.

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Jensen, Carl D. F.
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Peter, Philip A.

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Stevens, Ralph W.

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Kadin, Maurice

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Holmes, W. John
Pang, Herbert G.

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Buxeda, Roberto

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Marshall, M. R.
Wiggins, Robert L.

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Elvin, Norman L.

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Dyson, Charles

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Levitt, Herbert
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MacDonald, Roy K.
Ormsby, Hugh L.
Shusterman, Morris

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Cloutier, Roland
Locke, John C.
Viger, R. J.

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Mallek, Howard

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de Andrade, Cesario

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Dasilva, Antonio I.

SAO PAULO
Alvaro, Moacyr E.

CANAL ZONE
Fixott, Richard S.

CUBA

HAVANA
Ferrer, Horatio

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MANAGUA
Cuadra, Marie K.

PERU

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Barrere, Luciano E.
Raffo, Julio C.

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Velarde, Herminio, Sr.

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Quilala, Francisco V.

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GHENT
Francois, Jules

ENGLAND

LONDON
Duke-Elder, Lady Phyllis
Duke-Elder, Sir Stewart

FINLAND

HELSINKI
Koistinen, Aune Marjatta

ITALY

PARMA
Grignolo, Antonio

SOUTH AFRICA

JOHANNESBURG
Taylor, Israel B.

SWITZERLAND

GENEVA
Blum, John

TURKEY

ISTANBUL
Sezer, F. Necolet

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MARAVAL, TRINIDAD
Chan-Pong Norman R.